Moraxella lincolnii sp. nov., Isolated from the Human Respiratory Tract, and Reevaluation of the Taxonomic Position of *Moraxella osloensis*

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A polyphasic taxonomic study was performed to determine the relationships of 10 *Moraxella*-like strains isolated mainly from the human respiratory tract in Sweden. Two of the strains formed a separate subgroup on the basis of both their protein contents and their fatty acid contents. However, the overall protein and fatty acid profiles revealed that all 10 strains were highly related. Representative strains of the two subgroups exhibited high DNA binding values (98%) with each other and had an identical DNA base ratio (44 mol% G+C). DNA-rRNA hybridizations revealed that this taxon can be included in the genus *Moraxella*, which is only distantly related to phenotypically similar genera, such as the genera *Neisseria* and *Kingella*. The results of an extensive phenotypic analysis indicated that the general biochemical profile of the 10 strains conforms with the description of the genus *Moraxella* given in *Bergey's Manual of Systematic Bacteriology*. We therefore consider these organisms members of a new *Moraxella* species, for which the name *Moraxella lincolnii* is proposed. Furthermore, we also conclude that *Moraxella osloensis* belongs, genotypically as well as phenotypically, to the genus *Moraxella*.

Between 1980 and 1989, 10 *Moraxella*-like strains were isolated in Sweden, mainly from the human respiratory tract. In October 1980, immunotyping (Ouchterlony immunodiffusion and immunoelectrophoresis) was performed on the first three isolates, and the results revealed that the strains represented a distinct taxon which was provisionally named "*Moraxella lincolnii*." Subsequent isolates were rapidly identified. In the present study we used a polyphasic approach to determine the phenotypic and genotypic affiliation of these organisms. We found that the 10 strains constitute a new species belonging to the genus *Moraxella*, and we propose the name *Moraxella lincolnii* sp. nov. This name is used below. In view of the new data and recently published results (9), we also reevaluated the taxonomic position of *Moraxella osloensis* as the arguments used by Rossau et al. to remove this species from the genus *Moraxella* (18) were not conclusive.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All of the strains used are listed in Table 1. We obtained weak growth of *M. lincolnii* strains on several blood agar bases, including 5% (vol/vol) horse blood in Mueller-Hinton agar (catalog no. CM337; Oxoid, Ltd., Basingstoke, United Kingdom), nutrient agar no. 2 (catalog no. CM67; Oxoid), Columbia agar (catalog no. CM331; Oxoid), and heart infusion agar (catalog no. 0038-01-5; Difco Laboratories, Detroit, Mich.). Growth was not markedly enhanced on chocolate agar plates. The best growth results were obtained on a medium routinely used in our department for the cultivation of fastidious *Campylobacter* strains. This medium contains (per liter) 10 g

of special peptone (catalog no. L72; Oxoid), 5 g of Lab Lemco powder (catalog no. L29; Oxoid), 5 g of yeast extract (catalog no. L21; Oxoid), 5 g of sodium chloride, 2 g of sodium succinate hexahydrate, 2 g of sodium L-glutamate monohydrate, 1 g of magnesium chloride hexahydrate, and 15 g of agar no. 3 (catalog no. L13; Oxoid); the final pH is 7.0. This medium was supplemented with 5% (vol/vol) horse blood and is referred to below as standard medium. Optimal growth results were obtained at 28°C in a microaerobic atmosphere containing 5% O₂, 10% CO₂, and 85% N₂. *M. osloensis* strains and reference strains belonging to other *Moraxella* species were cultivated aerobically at 28°C on heart infusion agar (Difco).

Phenotypic tests. Phenotypic tests were performed on all *M. lincolnii* strains and on *Moraxella* reference strains. The morphology of cells was evaluated by Gram staining. Motility was observed in young cultures by examining wet mounts in broth by phase-contrast microscopy. The following tests were performed as described previously (5): presence of oxidase, catalase, β -galactosidase, DNase, and urease; production of indole; reduction of nitrate and nitrite; denitrification; growth under anaerobic conditions; growth on Drigalski agar; growth at different temperatures; growth in the presence of different concentrations of NaCl; oxidation or fermentation of D-glucose; liquefaction of gelatin; proteolysis on Löffler slants; hydrolysis of Tween 80; tolerance of penicillin (10- μ g discs); hemolysis and odor on horse blood agar; and formation of pigment on nutrient agar. Acid production from D-glucose, maltose, D-fructose, and sucrose was evaluated in a medium containing 15 g of Proteose Peptone no. 3 (catalog no. 0122; Difco), 4.5 g of NaCl, 3.0 g of Na₂HPO₄ · 2H₂O, 1.5 g of sodium alginate, and 17 g of Bacto Agar (catalog no. 0140; Difco) in 1,000 ml of placenta extract (the pH was adjusted to 7.7). This basal medium was supplemented with 2.5 ml of phenol red (0.5% solution), 5 ml of hemin (0.2% solution), 90 ml of ascitic fluid, and 10 ml of

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TABLE 1. Strains used

Taxon	Strain ^a	Received from ^a :	Source, if known
<i>M. lincolnii</i>	CCUG 9405 ^T (= LMG 5127 ^T)	Lincoln	Human nasopharynx, 6-mo-old female with fever (1980, Sweden)
<i>M. lincolnii</i>	CCUG 9406 (= LMG 10487)	Lincoln	Human throat, 1.5-yr-old male, respiratory tract infection (1980, Sweden)
<i>M. lincolnii</i>	CCUG 9511 (= LMG 10488)	Lange	Human nasopharynx, 1.5-yr-old female (1980, Sweden)
<i>M. lincolnii</i>	CCUG 11973 (= LMG 10489)	Dennford	Human nasopharynx, 2-yr-old child, whooping cough specimen (1982, Sweden)
<i>M. lincolnii</i>	CCUG 17467 (= LMG 10490)	PHL	Human nasopharynx, 21-yr-old female (1985, Sweden)
<i>M. lincolnii</i>	CCUG 18789 (= LMG 10491)	Dennford	Human eye, 5-mo-old male (1986, Sweden)
<i>M. lincolnii</i>	CCUG 20072 (= LMG 10492)	Brönnestam	Human respiratory tract, 11-mo-old male (1987, Sweden)
<i>M. lincolnii</i>	CCUG 20073 (= LMG 10493)	Brönnestam	Human nasopharynx, 1-yr-old male (1987, Sweden)
<i>M. lincolnii</i>	CCUG 21674 (= LMG 10494)	PHL	Human nasopharynx, 6.5-yr-old female (1987, Sweden)
<i>M. lincolnii</i>	CCUG 24769 (= LMG 10495)	PHL	Human blood, bursitis of the left knee (1989, Sweden)
<i>M. osloensis</i>	LMG 987 (= ATCC 10973)	TRS	
<i>M. osloensis</i>	LMG 1043 (= ATCC 19961 = NCTC 10756)	TRS	Human urine (1951, Norway)
<i>M. osloensis</i>	LMG 1044 (= ATCC 19963 = NCTC 10749)	TRS	Human nose (1951, Norway)
<i>M. osloensis</i>	CCUG 63 (= LMG 6916)	Lincoln	Human urine, 10-yr-old girl, suspected pyelonephritis (1968, Sweden)
<i>M. osloensis</i>	CCUG 350 ^T (= LMG 5131 ^T = ATCC 19976 ^T)	Bøvre	Human cerebrospinal fluid
<i>M. osloensis</i>	CCUG 11977 (= LMG 6917)	PHL	Human blood, 82-yr-old male with pneumonia (1982, Sweden)
<i>M. osloensis</i>	CCUG 13305 (= LMG 6918)	PHL	Human abdomen, 15-yr-old female, pus from appendix (1983, Sweden)
<i>M. osloensis</i>	CCUG 13369 (= LMG 6919)	PHL	Human blood, 8-yr-old male (1983, Sweden)
<i>M. osloensis</i>	CCUG 13705 (= LMG 6920)	PHL	Human cerebrospinal fluid, 24-yr-old female, meningitis (1983, Sweden)
<i>M. atlantae</i>	CCUG 6415 ^T (= LMG 5133 ^T = ATCC 29525 ^T)	Bøvre	Human blood (United States)
<i>M. bovis</i>	CCUG 2133 ^T (= LMG 986 ^T = ATCC 10900 ^T)	Bøvre	Cattle, pinkeye
<i>M. canis</i>	CCUG 8415A ^T (= LMG 11194 ^T)	PHL	Human wound, 29-yr-old woman, dog bite (1979, Sweden)
<i>M. catarrhalis</i>	CCUG 353 ^T (= LMG 11192 ^T = ATCC 25238 ^T)	Bøvre	
<i>M. caviae</i>	CCUG 355 ^T (= LMG 5129 ^T = ATCC 14659 ^T = CCUG 2132 ^T)	Bøvre	Pharynx, guinea pig (United States)
<i>M. cuniculi</i>	CCUG 2154 ^T (= LMG 8382 ^T = ATCC 14688 ^T)	Berger	Rabbit oral mucosa
<i>M. lacunata</i>	CCUG 4441 ^T (= LMG 5301 ^T = CIP A182 ^T)	CIP	Subacute conjunctivitis
<i>M. lacunata</i>	LMG 1009 (= ATCC 17952 = CCUG 4862 = NCTC 7911)	TRS	
<i>M. nonliquefaciens</i>	CCUG 348 ^T (= ATCC 19975 ^T = NCTC 10464 ^T)	Bøvre	Nose swab
<i>M. nonliquefaciens</i>	LMG 1011 (= ATCC 17954)	TRS	Human throat swab (1950, Canada)
<i>M. ovis</i>	CCUG 354 ^T (= LMG 8381 ^T = ATCC 33078 ^T)	Bøvre	Ovine eye, conjunctivitis

^a ATCC, American Type Culture Collection, Rockville, Md.; Berger, U. Berger, Heidelberg, Germany; Brönnestam, R. Brönnestam, Skovde, Sweden; Bøvre, K. Bøvre, Oslo, Norway; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; CIP, Collection de l'Institut Pasteur, Paris, France; Dennford, J. Dennford, Borås, Sweden; Lange, S. Lange, Göteborg, Sweden; LMG, Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium; Lincoln, K. Lincoln, Göteborg, Sweden; NCTC, National Collection of Type Cultures, London, United Kingdom; PHL, Public Health Laboratory Service, Göteborg, Sweden; TRS, Torry Research Station, Aberdeen, Scotland.

a 30% carbohydrate solution. API ZYM tests were performed according to the recommendations of the manufacturer (API bioMérieux, La Balme-les-Grottes, Montalieu-Vercieu, France).

The following enzymes belonging to a noncommercial API gallery were found to be useful for differentiation of *Moraxella* species: γ -glutamyl arylamidase, glycine aryl-

amidase, lysine arylamidase, proline arylamidase, α -glutamyl- α -glutamic acid arylamidase, glycyl-phenylalanine arylamidase, prolyl-arginine arylamidase, seryl-methionine arylamidase, and alanyl-phenylalanyl-prolyl-alanine arylamidase.

Gas chromatographic analysis of fatty acid methyl esters. Fatty acid compositions were determined after cultivation of

FIG. 1. Electrophoretic protein profiles and corresponding dendrogram derived from unweighted pair group average linkage of correlation coefficients for the protein patterns of all of the strains studied. The following molecular weight markers (track MWM) were used (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). The *M. lincolnii* strains marked with an asterisk were cultivated under the conditions used for *Moraxella* reference strains.

cells for 24 h on Trypticase soy agar (catalog no. 11768; BBL Microbiology Systems, Cockeysville, Md.) at 28°C. All *M. lincolnii* strains were incubated microaerobically. *M. osloensis* strains, as well as two representative *M. lincolnii* strains (strains CCUG 9405^T [T = type strain] and CCUG 18789), were incubated aerobically.

A loopful of well-grown cells was harvested, and fatty acid methyl esters were prepared, separated, and identified as described previously (22).

PAGE of whole-cell proteins. All *M. lincolnii* strains were cultivated under microaerobic conditions on standard medium. *M. osloensis* strains, all *Moraxella* reference strains, and three representative *M. lincolnii* strains (CCUG 9405^T, CCUG 17467, and CCUG 18789) were grown for 48 h on heart infusion agar (Difco) at 28°C. Whole-cell protein extracts were prepared, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE) was performed as described previously (13).

Numerical analysis of the protein gel electropherograms. A densitometric analysis, normalization and interpolation of the protein profiles, and a numerical analysis were performed by using the GelCompar software package (Applied Maths, Kortrijk, 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, converted for convenience to a percentage. The stacking gel-separation gel interface and the protein bands with a molecular weight below 22,000 (Fig. 1) were not used in the numerical analysis.

Preparation of high-molecular-weight DNAs. High-molecular-weight native DNAs of *M. lincolnii* strains were prepared

from 1- to 2-g portions of wet cells as described previously (22). DNAs from *Moraxella* reference strains and *M. osloensis* were available from previous studies (9, 18).

DNA-DNA hybridization experiments. The degree of DNA-DNA binding, expressed as a percentage, was determined spectrophotometrically by the initial renaturation rate method (4). Each value given below is the average of values from at least two hybridization experiments. DNA-DNA binding values of 30% and less indicate no significant DNA homology. The total DNA concentration was about 57 µg/ml, and the optimal renaturation temperature in 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) was 64.9°C.

DNA base compositions. The mean guanine-plus-cytosine (G+C) contents were determined by the thermal denaturation method and were calculated by using the equation of Marmur and Doty (14), as modified by De Ley (3).

DNA-rRNA hybridization experiments. In vivo radioactively labelled rRNA from *Moraxella lacunata* LMG 1009 was available in our research group. Fixation of single-stranded DNA on membrane filters, chemical determination of the amount of DNA on a filter, saturation hybridization, RNase treatment, and thermostability measurements of hybrids were performed as described by Van Landschoot and De Ley (23). Each DNA-rRNA hybrid was characterized by its $T_{m(e)}$ (melting temperature of elution), the temperature at which 50% of the hybrid was denatured. The higher the $T_{m(e)}$ of a heterologous hybrid, the closer it was related to the reference strain (homologous hybrid).

Immunotyping analysis. Preparation of antigens, immunization, and immunodiffusion were performed by the method of Falsen (6), as described previously (20). Antisera were

prepared against *M. lincolnii* CCUG 9405^T and CCUG 9511 and against reference strains of *M. osloensis*, *M. lacunata*, *Moraxella nonliquefaciens*, *Moraxella bovis*, *Moraxella ovis*, *Moraxella catarrhalis*, *Moraxella caviae*, and *Moraxella atlantae*. Antigens were prepared from all *M. lincolnii* strains and *Moraxella* reference strains.

RESULTS

Phenotypic analysis. The following features were present in all of the strains tested: oxidase activity, susceptibility to penicillin (10-μg discs), growth at 37°C, growth in a CO₂-enriched atmosphere, growth in the absence of NaCl, ester lipase C₈ activity, and leucine arylamidase activity. The following features were absent in all strains: motility, pigment production, odor production, anaerobic growth, oxidation or fermentation of glucose, denitrification, production of indole, urease activity, β-galactosidase activity, growth in the presence of 7.5 and 10% NaCl, production of acid from D-glucose, maltose, D-fructose, and sucrose, and lipase (C₁₄), valine arylamidase, cysteine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities. Tests that yielded variable results are shown in Table 2.

The type strains of all other *Moraxella* species were included as reference strains. Sometimes, the reactions of these type strains differed in some tests from the reactions of other strains belonging to the same species (7). For these tests, the general reaction pattern of the species is given in a footnote to Table 2. A number of discrepancies with our previous test results (9) were noted (*M. ovis* and *Moraxella cuniculi* strains produced DNase and *M. caviae* reduced nitrate in previous tests, whereas the opposite reactions were recorded in this study).

Fatty acid methyl ester composition. The fatty acid methyl ester compositions of all *M. lincolnii* and *M. osloensis* strains studied are shown in Table 3. Fatty acids which accounted for less than 1% of the total fatty acids in all of the strains studied are not given; therefore, the percentages in Table 3 do not always add up to 100%. *M. lincolnii* strains have 10:0, 12:0 3OH, 16:1 *cis*-9, 16:0, 18:1 *cis*-9, and 18:0 as their major components. *M. osloensis* strains have 10:0, 16:1 *cis*-9, and 18:1 *cis*-9 as their major components. The unidentified fatty acid methyl ester with an equivalent chain length of 15.490 was considered 14:0 3OH (15).

The fatty acid compositions of two *M. lincolnii* strains were considerably influenced by the composition of the gas atmosphere. The differences were most apparent in the ratio of 16:1 *cis*-9 to 18:1 *cis*-9, which was approximately 0.5 under microaerobic conditions and 2.0 under aerobic conditions.

Two *M. lincolnii* strains (CCUG 9511 and CCUG 11973) do not contain 12:0, a fatty acid which is present at an average level of $3.7 \pm 0.7\%$ in all other *M. lincolnii* strains. Below, these two strains are referred to as subgroup II strains, while subgroup I is used for the remaining eight strains, including the type strain.

The fatty acid compositions of *M. osloensis* strains are more heterogeneous than those of *M. lincolnii* strains, although so far no subgroups have been delineated. The general fatty acid profile of *M. osloensis* is similar to the profiles reported by Jantzen et al. (10) and Sugimoto et al. (19).

PAGE of whole-cell proteins. Duplicate protein extracts of several strains were prepared to check the reproducibility of

the growth conditions and the preparation of the extracts. The level of correlation between duplicate protein patterns was more than 95%.

Within *M. lincolnii*, two protein electrophoretic subgroups were found; the subgroup II strains (CCUG 9511 and CCUG 11973) have a profile which is considerably different from the profile of the other strains in the 34,000- to 45,000-molecular-weight region (Fig. 1). In the dendrogram obtained after numerical analysis of the protein patterns, we found a level of correlation of 79% between the two subgroups (Fig. 1). To allow a comparison of the protein profiles of the *M. lincolnii* strains with the protein profiles of other *Moraxella* species, we also cultivated three *M. lincolnii* strains under the conditions used for *Moraxella* reference strains (Fig. 1, asterisk). The protein patterns of the *Moraxella* strains cultivated under different conditions differ mainly in the density of some protein bands (Fig. 1). These patterns cluster together with a level of correlation of 77% (Fig. 1).

All *M. osloensis* strains group above a level of correlation of 74%. As Fig. 1 shows, the overall protein profiles of the *M. osloensis* strains are rather heterogeneous, although no subgroups can be delineated. The protein profiles of the other *Moraxella* reference strains are clearly different from the profiles of *M. lincolnii* strains and exhibit low correlation coefficients in comparisons with *M. lincolnii* and *M. osloensis* strains (less than 48 and 65%, respectively).

DNA-rRNA hybridization results. Representative strains belonging to subgroups I and II (CCUG 24769 and CCUG 9511, respectively) were chosen to perform DNA-rRNA hybridizations. We obtained $T_{m(e)}$ values of 74.4°C (CCUG 9511) and 74.8°C (CCUG 24769) with *M. lacunata* LMG 1009 rRNA. These values are quite low because of the gradual decomposition of the ³H-labelled rRNA during storage (9) and are comparable to the slightly higher values found previously (18) for *M. osloensis*.

DNA-DNA hybridization results. Representative strains belonging to subgroups I and II (CCUG 9405^T and CCUG 9511, respectively) were chosen to perform DNA-DNA hybridizations. These strains exhibited a high DNA-DNA binding value (98%) with each other, but the DNA-DNA binding values with reference strains of other *Moraxella* species, including *M. osloensis*, were not significant (less than 30%) (data not shown).

DNA base composition. The DNA base ratio of *M. lincolnii* CCUG 9511 and CCUG 24769 is 44 mol% G+C.

Immunotyping. All *M. lincolnii* strains exhibited high precipitation values with antisera prepared against the two *M. lincolnii* strains, while no significant precipitation values were recorded with antiserum prepared against any of the *Moraxella* reference strains (data not shown). Antigens from *Moraxella* reference strains did not cross-react with antiserum against *M. lincolnii* strains (data not shown).

DISCUSSION

The strains that we consider to be members of a new *Moraxella* species were obtained from diverse sources (Table 1). They were provisionally assigned to the genus *Moraxella* on the basis of their Gram reaction, cellular morphology, absence of motility, and reactions in a restricted set of biochemical tests. Their assignment to the family the *Moraxellaceae* was confirmed by DNA-rRNA hybridization data, which showed that these organisms are situated at the base of the *M. lacunata* rRNA homology group (18), which is phylogenetically far removed from phenotypically similar genera, such as the genera *Neisseria* and *Kingella* (9, 17, 18).

TABLE 2. Phenotypic features of *Moraxella* species

Feature	<i>M. lincolni</i> (10 strains)	<i>M. osloensis</i> CCUG 350 ^T	<i>M. nonliquefaciens</i> CCUG 348 ^T	<i>M. lacunata</i> CCUG 444 ^T	<i>M. atlantae</i> CCUG 6415 ^T	<i>M. catarrhalis</i> CCUG 353 ^T	<i>M. bovis</i> CCUG 2133 ^T	<i>M. ovis</i> CCUG 354 ^T	<i>M. cuniculi</i> CCUG 2154 ^T	<i>M. canis</i> CCUG 8415A ^T	<i>M. caviae</i> CCUG 355 ^T
Catalase activity	+	+	+	+	+	+	+	+	+	+	+
Hemolysis	-	-	-	-	-	-	-	-	-	-	-
DNase activity	-	-	-	NT	-	+	+	-	-	+	+
Growth on Drigalski agar	-	+	-	-	-	-	-	-	-	+	-
Growth at 42°C	-	+	+	-	-	-	-	+	+	+	+
Reduction of nitrate	-	-	+	+	-	+	+	+	-	+	+
Reduction of nitrite	d	-	-	-	-	+	+	-	-	-	+
Liquefaction of gelatin	-	-	-	+	-	-	+	-	-	-	-
Proteolysis on Löffler slants	-	-	-	+	-	-	+	-	-	-	-
Use of lactate as carbon source	-	+	-	-	-	-	-	-	-	+	-
Hydrolysis of Tween 80	-	-	-	-	-	-	-	-	+	-	-
Growth in the presence of 1.5% NaCl	-	+	+	+	+	+	+	+	+	+	+
Growth in the presence of 3% NaCl	-	-	+	-	W ^c	+	+	+	+	+	+
Growth in the presence of 5% NaCl	-	-	-	-	-	W ^d	-	+	-	+	-
Alkaline phosphatase activity	-	+	-	+	+	+	+	+	-	+	+
Esterase activity	+	+	+	+	+	+	+	+	-	+	-
Acid phosphatase activity	-	+	-	-	+	-	+	-	-	-	-
Phosphoamidase activity	-	+	-	+	+	-	-	-	-	+	-
γ-Glutamyl arylamidase activity	-	-	-	-	- ^d	-	-	-	-	+	-
Glycine arylamidase activity	+	+	+	+	- ^d	+	+	+	+	+	+
Lysine arylamidase activity	+	+	+	+	- ^d	+	+	+	+	+	+
Proline arylamidase activity	-	-	+	-	-	-	-	-	-	-	-
α-Glutamyl-α-glutamic acid arylamidase activity	d	-	+	- ^b	-	+	+	+	+	- ^d	+
Glycyl-phenylalanine arylamidase activity	d	+	+	+	-	- ^b	+	-	-	- ^b	- ^b
Propyl-arginine arylamidase activity	d	- ^b	+	- ^b	-	- ^d	+	+	-	- ^b	- ^b
Seryl-methionine arylamidase activity	+	+	+	+	-	+	+	+	-	+	+
Alanyl-phenylalanyl-propyl-alanine arylamidase activity	-	-	+	- ^b	-	+	+	-	-	+	- ^b

^a +, positive reaction; W, weak reaction; -, negative reaction; d, variable reaction depending on the strain tested; NT, not tested.^b Other strains of this species give variable reaction results (7).^c Other strains of this species give a negative reaction (7).^d Other strains of this species give a positive reaction (7).

TABLE 3. Average fatty acid compositions of *Moraxella* strains

Strain	% of:								
	10:0	12:0	12:0 3OH	14:0 3OH	16:1 <i>cis</i> -9	16:0	17:1 B ^a	18:1 <i>cis</i> -9	18:0
<i>M. lincolnii</i> : aerobic conditions									
CCUG 9405 ^T	9.0	5.5	9.5	— ^b	43.2	9.2	—	23.7	—
CCUG 18789	7.8	4.9	7.9	—	47.8	9.8	—	21.8	—
<i>M. lincolnii</i> : microaerobic conditions									
CCUG 9405 ^T	6.4	3.8	6.9	—	24.0	10.8	—	44.8	3.4
CCUG 9406	5.1	3.2	5.1	—	24.2	12.0	—	47.1	3.4
CCUG 9511	6.1	—	9.3	—	23.7	11.3	—	46.0	3.7
CCUG 11973	4.5	—	6.5	—	23.4	13.1	—	48.9	3.7
CCUG 17467	6.3	3.7	6.3	—	25.0	9.6	—	45.8	3.3
CCUG 18789	6.2	4.0	6.4	—	20.0	10.3	—	48.6	4.7
CCUG 20072	8.7	5.4	8.9	—	26.7	9.3	—	37.7	3.1
CCUG 20073	4.9	3.3	4.7	—	18.9	10.3	—	53.0	5.0
CCUG 21674	5.1	3.4	5.1	—	15.5	10.3	—	53.8	5.7
CCUG 24769	4.7	3.0	4.6	—	21.3	12.0	1.0	48.2	4.6
<i>M. osloensis</i>									
LMG 987	—	—	3.1	1.8	19.4	8.0	—	61.5	6.2
LMG 1043	7.4	—	4.0	1.7	19.3	3.4	2.2	59.7	2.4
LMG 1044	4.7	—	2.8	1.2	21.9	2.8	1.4	61.5	3.5
CCUG 63 ^c	5.1	—	2.6	1.3	38.9	3.6	1.1	43.7	1.3
CCUG 350 ^T	5.6	—	3.0	1.3	16.7	4.3	0.8	62.3	6.0
CCUG 11977	4.1	—	2.5	1.0	15.4	2.1	1.3	70.2	3.6
CCUG 13305	5.3	—	3.1	—	17.0	—	—	72.9	1.7
CCUG 13369	6.2	—	3.8	1.7	22.7	1.5	1.8	61.1	1.1
CCUG 13705	7.2	—	4.0	1.9	20.0	2.5	2.0	59.7	2.8

^a B, position of the double bond has not been determined.^b —, Absent or present in trace amounts (less than 1%).^c An unidentified fatty acid methyl ester with an equivalent chain length of 17.825 was also detected at a level of 2.5%.

Species belonging to the *M. lacunata* rRNA homology group (*M. lacunata*, *M. nonliquefaciens*, *M. bovis*, *M. catarrhalis*, *M. ovis*, *M. caviae*, *M. cuniculi*, and *Moraxella canis*) exhibit $T_{m(e)}$ values between 80 and 75.4°C with *M. lacunata* rRNA (9, 18), while $T_{m(e)}$ values of 74.8 and 74.4°C with the same rRNA were measured for two strains belonging to the new taxon (CCUG 24769 and CCUG 9511). Mainly on the basis of analogous $T_{m(e)}$ values we proposed previously that *M. osloensis* should be placed in a separate taxon since we restricted the genus *Moraxella* to members of the *M. lacunata* subgroup (18). Although differences in $T_{m(e)}$ of 5°C are commonly found within genera (8, 12, 16, 21, 24), we made this decision mainly because there was a clear difference between the $T_{m(e)}$ values of *M. osloensis* (75.5 to 75.7°C [18]) and the $T_{m(e)}$ values of members of the *M. lacunata* subgroup (76.3 to 80.0°C [18]). Now, we include *M. osloensis* and the new taxon in the genus *Moraxella* because the $T_{m(e)}$ values of these two groups (75.5 to 75.7°C [18] and 74.4 to 74.8°C, respectively) and the $T_{m(e)}$ values of the other *Moraxella* species (75.4 to 80.0°C [9, 18]) overlap when the experimental deviation of 0.5°C is taken into account. Whether organisms exhibiting differences in $T_{m(e)}$ values of about 5°C should be included in a single genus depends on additional phenotypic and chemotaxonomic parameters. For *M. osloensis*, the phenotypic and chemotaxonomic similarities with all other *Moraxella* species, including *M. canis* (9), are more decisive than the few distinct features mentioned previously (2, 10, 11, 17, 18). This is because the latter were based on a comparison of *M. osloensis* with only one or a few other *Moraxella* species. Furthermore, the general phenotypic profiles of *M. osloensis* and the new taxon for which we propose the name *M. lincolnii* and the DNA base ratios of

these organisms are consistent with the description of the genus *Moraxella* given in *Bergey's Manual of Systematic Bacteriology* (1) (Table 2). We therefore conclude that both *M. lincolnii* and *M. osloensis* are genuine members, genotypically as well as phenotypically, of the genus *Moraxella*.

M. lincolnii can easily be differentiated from other moraxellas by its lack of growth in the presence of 1.5% NaCl. Furthermore, the combined results obtained for alkaline phosphatase activity, reduction of nitrate, and growth at 42°C (Table 2) also allow differentiation of *M. lincolnii* from all other *Moraxella* taxa. Additional phenotypic tests useful for the differentiation of *Moraxella* species are given in Table 2. As shown in Fig. 1, analysis of the cellular protein content also allows clear-cut differentiation between *M. lincolnii* and allied taxa. Although two subgroups could be recognized on the basis of both protein contents and fatty acid contents, the overall profiles revealed that all 10 *M. lincolnii* strains were very similar. Representative strains of the two subgroups exhibited high DNA-DNA binding values (Fig. 1 and Table 2), while the DNA-DNA binding values between *M. lincolnii* strains and representatives of the *M. lacunata* rRNA homology group, including *M. osloensis*, were not significant.

Description of *Moraxella lincolnii* sp. nov. *Moraxella lincolnii* (lin'col.ni.i. N. L. gen. n. *lincolnii*, of Lincoln, in honor of K. Lincoln, a Swedish microbiologist, in recognition of his many contributions in the field of urinary tract infections). Cells are gram-negative, nonmotile, coccus-like to plump rods 1 to 1.5 µm wide and 1.5 to 2.5 µm long. The cells often occur in pairs and may form short chains. After 2 days of incubation, colonies are whitish, smooth, convex,

and circular and have a diameter of 1 to 3 mm. The colonies of some strains may have a flattened edge. No hemolysis and no production of pigment or odor. Grows under aerobic, capnophilic, or microaerobic conditions but not anaerobically. Grows on blood agar or nutrient agar, but not on Drigalski agar. Optimal growth occurs at 28 to 33°C. Growth also occurs at 36 to 37°C, but not at 42°C. Growth occurs in the absence of NaCl; no growth occurs in the presence of 1.5% NaCl. Oxidase and catalase activities are present. All strains are susceptible to penicillin (10-μg discs). Most strains reduce nitrites (seven of nine strains tested). No fermentation or oxidation of D-glucose. No acid production from D-glucose, maltose, D-fructose, or sucrose. Lactate cannot be used as a carbon source. No denitrification, liquefaction of gelatin, proteolysis on Löffler slants, hydrolysis of Tween 80, or indole production. No urease, DNase, or β-galactosidase activity. Nitrate is not reduced.

Ester lipase C₈, esterase, leucine arylamidase, glycine arylamidase, lysine arylamidase, and seryl-methionine arylamidase activities are present; alkaline and acid phosphatase, phosphoamidase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, lipase (C₁₄), valine arylamidase, cysteine arylamidase, γ-glutamyl arylamidase, proline arylamidase, and alanyl-phenylalanyl-prolyl-alanine arylamidase activities are absent.

The DNA base composition of *M. lincolnii* is 44 mol% G+C. The major fatty acids are 10:0, 12:0 3OH, 16:1 *cis*-9, 16:0, 18:1 *cis*-9, and 18:0.

So far, *M. lincolnii* has been isolated mainly from the respiratory tract of humans. Its clinical significance is unknown.

The type strain is CCUG 9405 (= LMG 5127), which was isolated in 1980 in Göteborg, Sweden, from the nasopharynx of a 5-year-old girl with a fever. All *M. lincolnii* strains have been deposited in the Culture Collection of the University of Göteborg and the Culture Collection of the Laboratorium voor Microbiologie, University of Ghent.

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