Moraxella atlantae sp. nov. and Its Distinction from *Moraxella phenylpyrouvica*

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A homogeneous group of gram-negative, rod-shaped, and oxidase-positive bacterial strains, growing with small colonies, is described. In ordinary cultural-biochemical tests, the entity appeared most similar to *Moraxella phenylpyrouvica*, although it has so far been distinguished from that species by its simultaneous lack of urease and phenylalanine and tryptophan deaminase activities, inability to grow on solid medium at 4 to 10°C, relative salt sensitivity, and different behaviour on bile-containing media. The fatty acid composition was very similar to that of *M. phenylpyrouvica*, but the organism was distinguishable from the latter in containing a fatty alcohol, *n*-octadecanol, and mannose. Its genetic affinity to *M. phenylpyrouvica* and other recognized *Moraxella* species appeared very low, but was detectable by transformation. The guanine plus cytosine content of its deoxyribonucleic acid was 46.0 to 47.5 mol%, which is 2.5 to 4.0% higher than in *M. phenylpyrouvica*. The entity is considered a new species of genus *Moraxella*, and the *Moraxella atlantae* is proposed.

The late Elizabeth O. King, Communicable Disease Center, Atlanta, Ga., provided us with a number of oxidase-positive, rod-shaped bacterial strains of unknown relation to *Moraxella*. On genetic and other bases, several of the strains were later allocated to three newly created species, *Moraxella osloensis* (3, 8), *M. phenylpyrouvica* (9), and *Kingella kingae* (11, 16, 18). The material received from King also contained a group of strains that was urease negative (named group 3 by her; see also reference 24), but was otherwise very similar to urease-positive strains later found to belong to *M. phenylpyrouvica*. Four strains of her group 3 and a similar strain isolated in Oslo were, in the present study, examined in detail and compared to *M. phenylpyrouvica*. Initially, the affinities of the strains were studied by genetic transformation and determination of deoxyribonucleic acid (DNA) base composition. Subsequently, the genetically defined groups were compared phenotypically.

MATERIALS AND METHODS

Bacterial strains. Four strains were received from the late Elizabeth O. King, Atlanta, Ga. Their CDC (Communicable Disease Center) numbers are A279, A1922, 5118, and 8330. Strain B19941/75 was received from Tone Skarpaas, National Institute of Public Health, Oslo, Norway. All strains had been isolated from human clinical material—four from blood and one (strain 8330) from spleen.

The following strains of *M. phenylpyrouvica* were included for comparison: 240, A390, 752/52, A1019 = ATCC 23335, A1232, 2863 = ATCC 23333 = NCTC 10526 (type strain), 5542 = ATCC 23334, 9158, 9413, 11865/52 = ATCC 19991, and ATCC 17950. Strain 240 was received from U. Berger, Hygiene-Institut der Universität, Heidelberg, Germany, and strain 11865/62 was from S. D. Henriksen, Kaptein W. Wilhelmsen og Frues Bakteriologiske Institutt, University of Oslo, Norway. The other strains were included in the original description of *M. phenylpyrouvica* (9).

The genetic transformation experiments included strains of all known species of *Moraxella*, *Kingella*, *Acinetobacter* and several *Neisseria* species (see Tables 2 and 3). Their mutual genetic relations were studied previously (references of Tables 2 and 3).

Genetic transformation. The procedures of mutant selection, preparation of donor DNA, and quantitative transformation with streptomycin resistance as the genetic marker were the same as those used before (7). Resistant mutants were selected in a one-step procedure on blood agar plates at a concentration of 500 µg/ml of streptomycin. The DNA exposure was terminated after 20 min by deoxyribonuclease. Assay of transformants were performed at a streptomycin concentration of 50 µg/ml, to insure uncritical conditions for phenotypic expression. In addition, the sensitive technique of long-term DNA exposure on plates during growth (7) was used when estimating genetic relationships between species or groups.

Determination of DNA base composition. DNA was prepared and its buoyant density in CsCl gradients was determined in an analytical ultracentri-

fuge as previously described (5). The density was measured versus *Escherichia coli* K-12 DNA, considered traditionally to have the density 1.710 g/cm³. The average DNA base composition, i.e., the moles percentage of guanine plus cytosine (mol% G+C), was calculated from the buoyant density, using the simplified formula mol% G+C = 1,000 ($\rho - 1.660$), where ρ = buoyant density in g/cm³ (5). Two separate experiments were performed, including the strains A279, A1922, 5118, and two *M. phenylpyruvica* controls of known DNA base composition (see Table 4). In experiment b, Table 4, the analytical ultracentrifugation was kindly performed by M. Fiandt, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wis.

Cultural and associated procedures. The characterization was based on the methodology proposed as Minimal Standards for Description of New Taxa in the Genera *Moraxella* and *Acinetobacter* (10). Examination for poly- β -hydroxybutyrate inclusions was performed after 20 h of growth on 10% horse serum nutrient agar containing 0.5% DL- β -hydroxybutyrate, as well as from Hutner medium when growth occurred. Agar corrosion and colony spreading were generally studied on freshly prepared human blood agar plates (6) incubated in a humid atmosphere. Colonies were photographed in incident light, except when examining for twitching motility (15); in the latter case, cultures on tryptose blood agar base (Oxoid) were studied by transmitted light phase-contrast microscopy, using a long focal distance condenser and a dry objective. H₂S production was tested for on TSI medium. Salt tolerance was studied by growth at different concentrations of sodium chloride incorporated in blood agar medium, and bile salt stimulation and tolerance were studied after incorporation of bile salts (Oxoid, code L55) in plates of tryptose blood agar base (Oxoid). When not

particularly mentioned, the incubation temperature was 33°C.

Negative-stain electron microscopy. The negative-stain electron microscopy techniques were generally as described (6). Cells were scraped off blood agar plates after 11 and 24 h of growth (humid atmosphere) and suspended in 0.8% (wt/vol) sodium silicotungstate (pH 7). During microscopy, a long focal distance holder for increased contrast was used in some instances.

Gas chromatography of whole cells. Fatty acids and the alcohol 18:0alc (see Results) were identified and quantitated by cultural, chemical, and gas chromatographic procedures previously described (19, 21), with few modifications. Lyophilized bacterial cells (10 mg) were treated with 2 N HCl in anhydrous methanol. After centrifugation, components of the supernatants were derivatized by trifluoroacetic anhydride, and the fatty acid methyl esters (including fatty alcohol) were selectively extracted by hexane. The analyses were performed on a Hewlett-Packard 5830A gas chromatograph, equipped with a flame ionization detector. Two glass columns (2 m long, 2 mm inner diameter) were used, with either 10% UCC-W982 (Hewlett-Packard) (unpolar) or 10% EGA (Supelco Inc.) (polar) on Gas-Chrom Q (100 to 120 mesh). Flow rate of carrier gas (N₂) was 20 ml/min. The temperature programme for the unpolar column started at 120°C and was increased by 2 degrees per min up to 270°C; for the polar column the temperature was increased from 140° to 200°C, by increments of 2 degrees per min, and then kept at 200°C for 20 min. The amount of each component is given (see Table 6) as the percentage of the total fatty acid (plus alcohol) content as determined from analysis on the unpolar column.

Gas chromatographic monosaccharide analysis of

TABLE 1. Quantitative streptomycin resistance transformation^a, showing intraspecies affinities between strains A279, A1922, 5118, 8330 and B19941/75

Expt no. ^b	Recipient strain	Donor strain	Transformant yield (T/E) ^c	Ratio of interstrain to autologous transformation
1	A1922	A1922	1.5×10^{-2}	
		A279	1.1×10^{-2}	7.3×10^{-1}
		5118	1.0×10^{-2}	6.7×10^{-1}
		8330	1.2×10^{-2}	8.0×10^{-1}
2	5118	5118	5.3×10^{-4}	
		A279	2.5×10^{-4}	4.7×10^{-1}
		A1922	3.5×10^{-4}	6.6×10^{-1}
3	5118	5118	3.0×10^{-4}	
		8330	1.7×10^{-4}	5.7×10^{-1}
4	A1922	A1922	1.7×10^{-2}	
		B19941/75	9.4×10^{-3}	5.5×10^{-1}

^a Short-term DNA exposure technique as previously described (7).

^b Each experiment was performed with identical aliquots of the same recipient population, exposed to the various donor DNAs in parallel.

^c T, Transformants; E, colony-forming units exposed to DNA.

^d Autologous transformation = transformation of the recipient with its own mutant DNA (first line of each experiment).

TABLE 2. Attempts to transform strain A1922 with DNAs from various species of oxidase-positive rods^a

Expt no. ^b	Donor species/strain ^c	Transformant yield:		Ratio of interstrain to autologous transformation ^e
		Per ml in 20-min DNA exposure	On comparable surface area in long-term DNA exposure ^d	
4	Strain A1922 <i>Moraxella phenylpyrouvica</i> 752/52	3.0×10^6 $\sim 1.0 \times 10^1$	Confluent 27	$\sim 3.3 \times 10^{-6f}$
5	<i>M. phenylpyrouvica</i> 752/52 <i>M. phenylpyrouvica</i> 2863, 11865/52, ATCC 17958		28 0-3	$< 10^{-6g}$
6	<i>M. phenylpyrouvica</i> 752/52 <i>M. nonliquefaciens</i> 4663/62 <i>M. bovis</i> ATCC 10900 <i>M. lacunata</i> ATCC 11748 <i>M. osloensis</i> A1920 <i>Kingella kingae</i> 4177/66 "M. urethralis" WM20 "New <i>Moraxella</i> " of Bijsterveld (1)		11 0-3	$< 10^{-6g}$

^a Streptomycin resistance transformation, performed as described (7).^b Each experiment was performed with identical aliquots of the recipient, exposed in parallel to different donor DNAs.^c All strains have been employed in previous taxonomic studies (1, 4, 5, 9, 11, 14, 16, 18, 19, 22).^d Technique as described previously (7).^e Autologous transformation = transformation of strain A1922 with its own mutant DNA (experiment no. 4, first line).^f Directly calculated from column 3.^g Calculated from the number of transformants in column 4 relative to the number elicited by strain 752/52.

the strains studied has been described separately (21a).

RESULTS

Genetic affinities in transformation. Table 1 shows the mutual affinities within the new group of strains. The ratios of interstrain to autologous transformation ranged from 4.7×10^{-1} to 8.0×10^{-1} , i.e., from 47 to 80%. These values are uniformly within the limits expected for strains belonging to the same species. For comparison, the ranges found in large groups of *M. nonliquefaciens* and *M. osloensis* strains were 34 to 99% and 32 to 100%, respectively (2, 3).

As shown in Tables 2 and 3, the new genetic entity had no close affiliation to any other recognized species of *Moraxella*, or to some doubtful associates of that genus. On the other hand, there was some affinity to well-established *Moraxella* species, such as *M. nonliquefaciens* and *M. osloensis*, with interstrain to autologous transformation reactions at the level of 10^{-5} to less than 10^{-6} , similar to reactions with the "false neisseriae" species *Neisseria catarrhalis* and *Neisseria ovis*. (The genus designa-

tion *Moraxella* [17] has been proposed for these organisms, and for one of them, *N. catarrhalis*, the genus name *Branhamella* has been proposed [13]. While this taxonomic problem is being reexamined, the designation *Neisseria* is maintained.) These interactions were in the same order of magnitude as that typically found between species in this large genetic group (Table 3 [4]). It is noted that one *M. phenylpyrouvica* strain was able to transform an active recipient of the new entity very weakly. On the other hand, no affinity was found to *Neisseria elongata* nor to an *Acinetobacter* strain.

DNA base composition. Table 4 shows that three strains of the new entity had G+C values for their DNAs ranging from 46 to 47.5 mol%. This range is clearly higher than that in strictly compared *M. phenylpyrouvica* and other established *Moraxella* species and "false neisseriae" (40 to 45 mol%) (5) and close to that of the tentatively named *M. urethralis* (22) and the new *Moraxella* of Bijsterveld (1).

Cells, colonies, and motility. The five strains of the new species consisted of variably sized, often plump, diplococcobacillary to dis-

TABLE 3. Transforming effect of DNAs of the new species and reference donors on various recipients of *Neisseriaceae*^a

Expt no. ^b	Recipient species/strain ^c	Donor species/strain ^c	Transformants per plate in long-term DNA exposure ^d	Ratio of interstrain to autologous transformation ^e
8	<i>Moraxella nonliquefaciens</i> NCTC 7784	<i>M. osloensis</i> 5873	76	<10 ^{-6f}
		<i>M. phenylpyrouvica</i> 752/52, 2863, 11865/52, ATCC 17958	15-29	<10 ^{-6g}
		New species, strains A279, A1922, 5118, 8330	23-61	<10 ^{-6g}
9	<i>M. osloensis</i> 5873	<i>M. nonliquefaciens</i> NCTC 7784	161	6 × 10 ^{-6f}
		New species, strains A279, A1922, 5118, 8330	173-331	6 × 10 ⁻⁶ -6 × 10 ^{-5g}
10	<i>Neisseria catarrhalis</i> Ne 11	<i>M. bovis</i> ATCC 10900	1,600	3.7 × 10 ^{-5f}
		<i>M. phenylpyrouvica</i> 2863	326 }	5 × 10 ⁻⁶ -9 × 10 ^{-6g}
		New species, strains A279, A1922, 5118, 8330	196-410 }	
11	<i>N. ovis</i> 199/55	<i>M. osloensis</i> 5873	1,300	2.7 × 10 ^{-5f}
		<i>M. phenylpyrouvica</i> 2863	561 }	~10 ^{-5g}
		New species, strain A279	479 }	
12	<i>N. elongata</i> M2	<i>N. meningitidis</i> B8152/66	4,000	9.2 × 10 ^{-3f}
		New species, strain 5118	0	<3 × 10 ^{-6g}
13	<i>Acinetobacter calcoaceticus</i> Bd4	<i>Acinetobacter</i> ATCC 17985	3,000	9.2 × 10 ^{-4h}
		<i>M. phenylpyrouvica</i> A390, 752/52, 2863	0-3	<6 × 10 ^{-7g}
		New species, strain A279	2	

^a Streptomycin resistance transformation, performed as described (7).^b Each experiment was performed with identical aliquots of the recipient, exposed in parallel to the various donor DNAs.^c All heterologous species/strains have been employed in previous taxonomic studies (4, 5, 8, 9, 12, 14, 20). *Neisseria catarrhalis* and *N. ovis* have alternative designations—*Moraxella* (17) or *Branhamella* (13) (see text).^d Technique as described (7).^e Autologous transformation = transformation of the recipient in question by its own mutant DNA.^f Reference ratios, based directly or indirectly on quantitative transformation experiments, previous determination (4, 12).^g Ratios calculated from reference ratios on the basis of relative value in long-term DNA exposure (column 4).^h Reference ratio, based on quantitative transformation experiments in this study (short-term DNA exposure [7]).

tinctly rod-shaped cells (averaging 1.0 by 2.0 µm; see below), with little tendency to grow in longer chains. When influenced by penicillin in low concentrations (slightly inhibited growth around penicillin depots on blood agar), they behaved like most other rod-shaped bacteria with constant division plane, with elongation

as long threads of somewhat varying dimension, but without formation of fusiform cells as seen in most strains of *M. osloensis* (K. Bøvre, N. Hagen, B. P. Berdal, and E. Jantzen, *Acta Pathol. Microbiol. Scand. Sect. B*, in press) and sometimes in *M. phenylpyrouvica* (Table 5). The cells were gram negative, with some ten-

TABLE 4. Determination of DNA base composition of the new species and of *Moraxella phenylpyrovuica*

Expt ^a	Strain	Buoyant density (ρ) in CsCl (g/cm ³) ^b	Mol% G+C ^c	Mol% G+C in literature
a	A279	1.7065	46.5	
	5118	1.7070	47.0	
	<i>M. phenylpyrovuica</i>	1.7035	43.5	42.5 ^d
	11865/52			
b	A1922	1.7060	46.0	
	5118	1.7075	47.5	
	<i>M. phenylpyrovuica</i>	1.7035	43.5	43.5 ^e
	752/52			

^a In each experiment DNA extraction and purification were done in parallel, and the buoyant densities were determined in the same analytical centrifuge run (5).

^b Referred to *Escherichia coli* K-12 DNA, assumed density 1.710 g/cm³.

^c Calculated from the buoyant density, using the simplified formula mol% G+C = 1,000 ($\rho - 1.660$).

^d Chemical determination by Catlin and Cunningham (14).

^e Buoyant density determination by Bøvre et al. (5).

dency to retain the crystal violet stain. Capsules or poly- β -hydroxybutyrate inclusions were not formed (Table 5).

The colonies were slightly opaque and of small size on all media (see below and Table 5), generally a little smaller than *M. phenylpyrovuica* colonies. Usually, two main colony variants could be observed in each strain, one hemispherical with even outline (Fig. 1), the other slightly larger, more flat, or with irregular margin, and with a tendency to form a spreading zone (Fig. 2, variants observed in Fig. 1). When the colonies were removed, pitting or corrosion of the agar could be observed, generally more pronounced beneath the latter colony type (Fig. 2). Transition from the former to the latter type often occurred on prolonged serial subculture, even without attempts to select for this type of colony. Thus, the colony type variation may be different from that observed in several *Moraxella* species, where nonspreading, noncorroding colonies tend to be spontaneously enriched in subculture (6). Exceptional was strain B19941/75, which never showed spreading, thus resembling *M. phenylpyrovuica* (Table 5). The strains with the greatest tendency to form corroding colonies, A1922 and 5118, were those having the highest competence in genetic transformation (Table 1). To some extent this resembles the association between colony type and competence observed in other species of *Moraxella* (7). However, stably noncorroding and incompetent variants of these two strains were not observed, so that the association could not be critically tested.

Agar plate microscopy of spreading zones showed cells or small groups of cells scattered on the agar (Fig. 3). Slight movement of occasional cells could be detected. As this was different from swimming and Brownian move-

ment and the morphology of the spreading zone seemed typical, the mechanism of spreading was considered likely to be twitching motility (15). No swimming motility could be detected in fluid media or hanging drop preparations.

Electron microscopy. The electron micrograph of Fig. 4 illustrates the size and form variation of the cells in strain A1922. The cells had a convoluted surface. A radiating pattern was typical of the granular zone just outside the cells. In some cases (not shown), this could extend 1 μ m from the cells and seemed to contain thin fimbriae. Typical fimbriae of 6- to 8-nm width were found associated with 25 to 100% of the cells, there being one to four per cell. Their length varied from 0.5 to several micrometers. They were mostly single and straight or slightly curved, and were found in relation to all parts of the cell surface, but possibly with some preference for the end regions. All colony variants studied had fimbriae, and no clear difference was apparent in number or pattern for hemispherical and flat colonies (see above). Cell wall protrusions were present to a varying extent on most of the cells.

Biochemical reactions and growth. The oxidase (both methods of reference 10) and catalase reactions were uniformly strongly positive. There was no pigment production or hemolysis (only human blood tested), except that in old blood agar cultures the colonies appeared slightly pink and the medium often showed a slight greenish discoloration. No discoloration of heated blood agar was observed. All strains grew in nutrient broth and on agar media without serum, including Hugh and Leifson O/F medium (distinct fine growth along the stab in three passages) and tryptose blood agar base (Oxoid). Growth was not clearly improved by blood, heated blood, serum, or CO₂. Only occa-

TABLE 5. Some morphological and cultural-biochemical characteristics of strains belonging to the new species and to *Moraxella phenylpyrovuica*^a

Test, character	New species (5 strains)		<i>M. phenylpyrovuica</i> (11 strains)	
	Typical	Deviating (strains)	Typical	Deviating (strains)
Fusiform cells with penicillin	—		—	w(A390, 752/52, 5542), + (9158)
Poly- β -hydroxybutyrate inclusions	—		—	w(2863)
Colony diameter, 20 h (mm) ^b	0.1-0.3	0.5(A1922)	0.4-0.5	
Colony diameter, 48 h (mm) ^b	0.2-0.5	1.0(A1922)	0.9-1.0	
Agar corrosion, pitting ^b	w	+ (A1922, 5118)	—	w(752/52)
Colony spreading observed	+	— (B19941/75)	—	
Peptone water, 1% (wt/vol), growth	w	+ (A1922, 5118)	+	
Koser citrate, growth	—	w(8330)	—	+ (2863)
Hutner medium with acetate, growth	—	w(B19941/75)	—	+ (2863)
Growth at 4°C ^b	—		w(6-14 days)	
Growth at 10°C ^b	—		w(4-5 days)	
Growth at 22°C ^b	w(4 days) ^c		+ (1 day)	
Survival at 4°C ^b	1-2 weeks	4-6 weeks(A279, 8330)	16 weeks	
Survival at 33°C ^b	6-16 weeks	2 weeks(5118)	16 weeks	
Nitrate, reduction to nitrite	—		+	— (2863), w(240, 9413)
Urease production	—		+	— (11865/52, ATCC 17958)
Phenylalanine/tryptophan deaminase	—		+	
Benzylpenicillin, MIC (U/ml) ^c	0.1	0.05(A1922)	0.01-0.025	0.05(240, A390, A1019, 5542, 9413)
NaCl, MIC (g/100 ml) ^b	3		9	7.5(5542), 4.5(1232)
Bile salts, ^d growth stimulated by concentrations (g/100 ml)	0.2-1 ^f		0.05-4 ^f	
Bile salts, ^d MIC (g/100 ml)	3		5	

^a Procedures as described in ref. 10 and in text. Other results not tabulated: all strains of the two species were oxidase and catalase positive, grew on Hugh and Leifson O/F medium, did not grow at 42°C or anaerobically, were nonhemolytic and did not produce acid from carbohydrates, and did not reduce nitrite, liquefy serum or gelatin, or produce indole or H₂S; for further characteristics, see text.

^b Blood agar (6), humid atmosphere.

^c MIC = minimal inhibitory concentration, determined by dilution in solid medium (heated blood agar).

^d Bile salts (oxid, code L55) incorporated in tryptose blood agar base (Oxoid).

^e w, Weak growth or reaction. Time in parentheses indicates delay before distinct growth.

^f All strains were stimulated by all concentrations within the range.

sionally was growth improved by a humid atmosphere. No growth occurred anaerobically (H₂ + CO₂) or at 42°C (solid and fluid media). Optimum temperature for growth was 33 to 37°C. There was no acid production from glucose or other carbohydrates (as mentioned in reference 10). Nitrite was not reduced. Coagulated serum and gelatin were not liquefied, and indole or H₂S was not produced. All these reactions and growth results were shared by the strains of the new species and all of 11 simulta-

neously examined strains of *M. phenylpyrovuica* (see Materials and Methods). It should be noted that these results with *M. phenylpyrovuica* differ distinctly from some reactions reported by Snell et al. (23). Snell et al. could not detect growth in Hugh and Leifson medium, and recorded growth anaerobically. They also found that *M. phenylpyrovuica* grew at 42°C.

Table 5 shows the results of other tests with all 16 strains of the two species. Some traits appeared distinctive between the two, but in

several cases overlaps and weak reactions disturbed the distinction. The new species appeared to lack the ability of all *M. phenylpyrovica* strains to grow on solid medium at 4° to 10°C (23). However, the growth of *M. phenylpyrovica* was slow at these temperatures, and in fluid medium growth also occurred from the strains A279, 8330, and B19941/75 of the new species (not tabulated). The new species was also less tolerant than *M. phenylpyrovica* to

FIG. 1. Strain A1922. Weakly corroding, hemispherical to conical colonies with flat variants on blood agar incubated for 2 days. Magnification, $\times 3.7$.

FIG. 2. Strain A1922. Corroding, mainly flat and partly spreading colonies on blood agar incubated for 2 days. Magnification, $\times 3.7$.

FIG. 3. Strain A1922. Margin of spreading colony on tryptose blood agar base (Oxoid), incubated for 1 day. Magnification, $\times 255$.

sodium chloride (minimal inhibitory concentration 3% versus 4.5 to 9%). Likewise, the behavior on bile salt-containing media appeared to be distinguishing. It is of importance to note that the new species, like *M. phenylpyrovica* (23), was stimulated by bile. However, the stimulation took place up to a higher concentration of bile salts in *M. phenylpyrovica* (up to 4% versus 1% for the new species). When stimulated, the colonies of *M. phenylpyrovica* usually attained a much greater size than did colonies of the new species. The minimal concentration of bile salts inhibiting growth was invariably higher in *M. phenylpyrovica*, but this difference was also rather small (5% versus 3%), particularly for comparison between results obtained in different laboratories. Finally, all strains of the new species were phenylalanine and tryptophan deaminase negative, whereas all *M. phenylpyrovica* strains here examined were positive (see Discussion).

Fatty acid and alcohol content. Table 6 presents the fatty acid and alcohol composition of whole cells of the new species. The similarity of the five strains was striking, and confirmed the genetic finding of a very homogeneous taxonomic entity. All the 14 compounds detected were found in genus *Moraxella* previously (19). Included in Table 6 are the corresponding values for the 11 strains of *M. phenylpyrovica*. This species was very homogeneous in these terms, and the similarity between the two species was very high, as the relative amounts of 13 out of 15 compounds (3-OH-16:0, absent in both species, included) overlapped. One fatty acid (16:1) was consistently lower in the new species, but the most clearly distinguishing

FIG. 4. Strain A1922. Cells from flat colony type (Fig. 2) on blood agar incubated for 11 h. Electron microscopy by negative staining. Magnification, $\times 40,000$. Bar represents $0.1 \mu\text{m}$.

component was the fatty alcohol *n*-octadecanol (18:0alc), which was consistently absent in *M. phenylpyrovica*. The alcohol was identical to the previously unidentified compound x-5 (19) and was recently shown to be a wax ester constituent (K. Bryn, E. Jantzen, and K. Bøvre, unpublished data). The two strains A1922 and 9413, one of each species, illustrate the similarity in fatty acid composition between the two entities. As seen from the calculation ("score") presented in Table 6, no precise allocation to species could be performed if the fatty alcohol was excluded from the comparison.

Monosaccharide composition. Gas chromatographic and other chromatographic studies of monosaccharide composition disclosed the presence of mannose in all five strains of the new species and its absence in all of the 11 strains of *M. phenylpyrovica* and other rod-shaped *Moraxella* species simultaneously examined (21a).

DISCUSSION

The data on genetic transformation and DNA base composition show that the strains A279, A1922, 8330, and B19941/75 represent one homogeneous and separate group satisfying criteria of a species (Tables 1 to 4). Its place in the genus *Moraxella* is strongly supported by the transformation data. The affinity in heterologous transformation to other rod-shaped *Moraxella* species and "false neisseriae" is of the same magnitude as affinities among some of the latter (4). In addition, several phenotypic traits, including fatty acid composition (Table 6 [19, 20]), confirm this allocation to genus.

Phenotypically, the new species most closely resembles *M. phenylpyrovica*. They are both characterized by small colonies, which do not increase significantly in size on serum media or heated blood agar, and share several other properties in conventional tests (Results; Table

TABLE 6. Comparison of fatty acid and alcohol composition of the new species and *Moraxella phenylpyrouwica*^a

Strain ^b														Score ^c as		
	12:0	3-OH-12:0	14:1	14:0	3-OH-14:0	15:0	16:1	16:0	3-OH-16:0	17:1	18:0alc	18:1	18:0	New species	<i>M. phenylpyrowuica</i>	
New species																
A279	1.7	11.4	—	1.5	1.7	0.3	1.6	32.4	—	0.6	0.8	14.1	16.5	15.3	13	7
A1922	1.6	9.1	0.2	2.4	1.7	0.4	2.8	26.7	—	0.6	0.5	21.2	21.7	9.7	9	7
5118	1.4	11.3	—	2.0	2.0	0.4	1.8	33.8	—	0.9	1.2	17.7	17.7	11.9	12	5
8330	0.9	10.0	—	1.3	2.2	0.2	1.0	29.2	—	0.7	0.7	13.1	21.5	16.9	12	8
B19941/75	0.4	8.7	0.1	1.5	1.9	0.2	2.7	31.6	—	0.4	0.3	16.2	21.4	12.4	13	9
$\bar{x}^c$	1.20	10.10	0.06	1.74	1.90	0.30	1.98	30.74		0.64	0.70	15.66	19.76	13.24		
σ^c	0.54	1.24	0.09	0.45	0.21	0.10	0.76	2.81		0.18	0.34	3.31	2.47	2.85		
<i>M. phenylpyrowuica</i>																
240	1.5	11.5	0.4	0.7	1.6	0.4	8.6	11.6	—	0.6	0.2	28.3	29.4	3.6	4	10
A390	0.5	12.2	0.3	0.5	0.4	—	10.9	11.0	—	0.5	—	27.4	30.7	3.6	2	9
752/52	0.6	13.9	1.4	1.1	0.5	0.6	5.8	16.0	—	0.5	0.2	26.1	25.3	5.1	2	15
A1019	0.4	14.6	0.4	0.7	0.2	0.3	7.7	10.0	—	0.5	0.1	28.9	29.2	3.3	3	11
A1232	0.6	16.1	0.5	2.0	—	0.3	7.4	26.6	—	0.7	0.4	10.5	22.5	9.6	5	12
2863	0.6	6.7	2.0	0.5	0.4	0.2	12.0	10.0	—	0.7	Tr	30.6	30.5	3.1	3	7
5542	0.4	18.9	0.1	1.1	0.6	1.8	11.3	22.2	—	0.6	0.3	11.9	20.5	8.1	4	14
9158	1.0	16.5	—	0.7	0.3	0.7	13.7	19.6	—	0.8	0.3	12.9	24.4	7.3	5	12
9413	3.5	15.8	0.8	1.7	1.5	0.7	4.5	30.6	—	0.5	0.6	10.5	16.8	11.5	6	7
11865/52	0.6	13.5	0.3	1.0	Tr	0.4	9.4	22.1	—	0.6	0.3	16.0	23.0	9.1	4	15
ATCC 17958	2.2	20.1	—	1.0	Tr	Tr	12.9	16.1	—	0.7	0.2	15.9	23.0	6.5	4	11
$\bar{x}$	1.01	14.29	0.62	1.27	0.45	0.45	9.47	19.44	—	0.60	0.24	18.21	24.13	6.88		
σ	0.87	3.38	0.56	0.69	0.50	0.45	2.80	7.01	—	0.10	0.15	8.01	4.37	2.72		

^a See text and references 19 and 21 for experimental details. The following fatty acid designations are used: the figure before colon indicates number of carbon atoms in the chain, the one after colon the number of double bonds; the symbol 3-OH indicates a hydroxy group in position 3 relative to the carboxyl group. 18:0alc denotes a straight-chain saturated fatty alcohol (*n*-octadecanol). The concentrations are given as percentage of the total fatty acid (plus alcohol) content. A few consistently minor, unidentified compounds have been excluded from the table. Tr (trace) indicates less than 0.1%. —, Not detected.

^b See Materials and Methods for further information on the strains employed.

^c The mean value $\bar{x}$, standard deviation σ and the "confidence interval" $\bar{x} \pm \sigma$ have been calculated for each of the compounds for both species. "Score" indicates how many of the tabulated concentrations of a strain which fall inside the "confidence intervals" of "new species" and "*M. phenylpyrouwica*," respectively.

5). Furthermore, the two species are particularly similar in fatty acid composition (Table 6). However, a close comparison between the new species and *M. phenylpyrourica* reveals several more or less clearly distinguishing hallmarks. The inability of the new species to grow on solid medium at 4 to 10°C, and at 33 to 37°C, to grow well with 3% sodium chloride or 3% bile salts in the medium, appears distinct from *M. phenylpyrourica*. The differences between the two in these respects are not pronounced, however, so that direct comparisons with several controls may be necessary for the use of these characteristics in identification. The tendency of the new species to form corroding and/or spreading colony variants may also be used for a tentative diagnosis. Phenylalanine and tryptophan deaminase activities were consistently absent in the new species. However, these activities have also been found missing or very weak in some recently isolated *M. phenylpyrourica* strains (Bøvre et al., in press), rendering the tests somewhat questionable for the distinction. Finally, the new species can be distinguished from *M. phenylpyrourica* by its cellular content of the fatty alcohol *n*-octadecanol, and mannose, detectable by gas chromatography.

The ability of the new species to grow on Hugh and Leifson O/F medium, its negative nitrate reaction, its relatively low sensitivity to penicillin (Table 5), and the mentioned points of similarity to *M. phenylpyrourica* may serve to distinguish it from *M. nonliquefaciens* (2). Key phenotypic traits of *M. osloensis*, although somewhat variable (Bøvre et al., in press), should make the distinction from that species relatively easy. However, as more strains are examined of the new species and of related species, ranges of phenotypic variation are expected to increase. In selected cases of identification it may therefore be important to utilize the systems of genetic transformation and gas chromatographic analysis developed for these microorganisms.

We propose the name *Moraxella atlantae* for the new species recognizing the pioneer work of the late Elizabeth O. King of Atlanta, Ga. The strain 5118 is proposed as the type strain. The strain A1922 may be a useful reference strain, due to its high transformability. All five strains have been deposited in the American Type Culture Collection and in the National Collection of Type Cultures (Great Britain).

Condensed Description of *Moraxella atlantae* sp. nov.

Cellular morphology. Variably sized, often plump, diplococcobacillary to distinctly rod-shaped cells (average 1.0 by 2.0 μ m), with little

tendency to grow in longer chains. Often fimbriated. Gram negative, with some tendency to resist decolorization. Not encapsulated. Does not form poly- β -hydroxybutyrate inclusions.

Colony morphology. Slightly opaque, small colonies on all media (diameter on blood agar most often 0.1 to 0.3 mm after incubation for 20 h). Two main colony variants, one hemispherical with an even outline, the other more flat or with irregular margin, and with a tendency to form a spreading zone. Pitting of the agar, more pronounced beneath the latter colony variant.

Motility. No swimming motility. May show twitching.

Oxidase and catalase reactions. Strongly positive.

Pigment production and hemolysis. None.

Culture medium, growth stimulation and tolerance. Grows with low cell yield in nutrient broth and on agar media without serum, including Hugh and Leifson O/F medium. No significant stimulation by serum or heated blood. Weak growth in peptone water, and usually no growth in Koser citrate and Hutner medium with acetate. Stimulated by bile salts up to 1 g/100 ml. Minimal inhibitory concentration of bile—3 g/100 ml; of sodium chloride—3 g/100 ml, all in solid medium.

Atmosphere. Strict aerobe, not distinctly stimulated by CO₂ or humidity.

Temperature. No growth on solid medium at 4 to 10°C, slight growth at 22°C, optimum temperature for growth 33 to 37°C, no growth at 42°C.

Survival. Blood agar cultures remain viable for 1 to 6 weeks at 4°C and for 2 to 16 weeks at 33°C (humid atmosphere).

Carbohydrates. No acid production.

Nitrate and nitrite reduction. Negative.

Proteolytic enzymes. Coagulated serum and gelatin are not liquefied, and indole or H₂S are not produced. Urease, phenylalanine deaminase, and tryptophan deaminase reactions are negative.

Sensitivity to penicillin. Minimal inhibitory concentration 0.05 to 0.1 U/ml.

Fatty acid, alcohol, and monosaccharide composition. Fatty acid pattern similar to that of *M. phenylpyrourica*, but differs from that species in containing the fatty alcohol *n*-octadecanol. Distinguishable from other rod-shaped *Moraxella* species by its cellular content of mannose.

DNA base composition. 46 to 47.5 mol% G+C.

Genetic affinities. Often transformable. No transformation (streptomycin resistance marker) detected with DNA from *M. nonlique-*

faciens, *M. lacunata*, *M. bovis*, *M. osloensis*, or *Kingella kingae*. Marginal transformation with *M. phenylpyruvica* DNA. Low, but distinct donor activity in transformation of recipients of *M. nonliquefaciens*, *M. osloensis*, and *Neisseria* (*Moraxella*, *Branhamella*) *catarrhalis* and *ovis* (10^{-5} to less than 10^{-6} , interstrain to autologous transformation). No affinity to *N. elongata* or *Acinetobacter*.

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REPRINT REQUESTS

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LITERATURE CITED

1. Bijsterveld, O. P. van. 1970. New *Moraxella* strain isolated from angular conjunctivitis. *Appl. Microbiol.* 20:405-407.
2. Bøvre, K. 1964. Studies on transformation in *Moraxella* and organisms assumed to be related to *Moraxella*. 2. Quantitative transformation reactions between *Moraxella nonliquefaciens* strains, with streptomycin resistance marked DNA. *Acta Pathol. Microbiol. Scand.* 62:239-248.
3. Bøvre, K. 1965. Studies on transformation in *Moraxella* and organisms assumed to be related to *Moraxella*. 6. A distinct group of *Moraxella nonliquefaciens*-like organisms (the "19116/51" group). *Acta Pathol. Microbiol. Scand.* 65:641-652.
4. Bøvre, K. 1967. Studies on transformation in *Moraxella* and organisms assumed to be related to *Moraxella*. 7. Affinities between oxidase positive rods and neisseriae, as compared with group interactions on both sides. *Acta Pathol. Microbiol. Scand.* 69:92-108.
5. Bøvre, K., M. Fianndt, and W. Szybalski. 1969. DNA base composition of *Neisseria*, *Moraxella*, and *Acinetobacter*, as determined by measurement of buoyant density in CsCl gradients. *Can. J. Microbiol.* 15:335-338.
6. Bøvre, K., and L. O. Frøholm. 1972. Variation of colony morphology reflecting fimbriation in *Moraxella bovis* and two reference strains of *M. nonliquefaciens*. *Acta Pathol. Microbiol. Scand. Sect. B* 80:629-640.
7. Bøvre, K., and L. O. Frøholm. 1972. Competence in genetic transformation related to colony type and fimbriation in three species of *Moraxella*. *Acta Pathol. Microbiol. Scand. Sect. B* 80:649-659.
8. Bøvre, K., and S. D. Henriksen. 1967. A new *Moraxella* species, *Moraxella osloensis*, and a revised description of *Moraxella nonliquefaciens*. *Int. J. Syst. Bacteriol.* 17:127-135.
9. Bøvre, K., and S. D. Henriksen. 1967. A revised description of *Moraxella polymorpha* Flamm 1957, with a proposal of a new name, *Moraxella phenylpyruvica*, for this species. *Int. J. Syst. Bacteriol.* 26:92-96.
10. Bøvre, K., and S. D. Henriksen. 1976. Minimal standards for description of new taxa within the genera *Moraxella* and *Acinetobacter*: proposal by the Subcommittee on *Moraxella* and Allied Bacteria. *Int. J. Syst. Bacteriol.* 26:92-96.
11. Bøvre, K., S. D. Henriksen, and V. Jonsson. 1974. Correction of the specific epithet kingii in the combinations *Moraxella kingii* Henriksen and Bøvre 1968 and *Pseudomonas kingii* Jonsson 1970 to kingae. *Int. J. Syst. Bacteriol.* 24:307.
12. Bøvre, K., and E. Holtén. 1970. *Neisseria elongata* sp. nov., a rodshaped member of the genus *Neisseria*. Re-evaluation of cell shape as a criterion in classification. *J. Gen. Microbiol.* 60:67-75.
13. Catlin, B. W. 1970. Transfer of the organism named *Neisseria catarrhalis* to *Branhamella* gen. nov. *Int. J. Syst. Bacteriol.* 20:149-150.
14. Catlin, B. W., and L. S. Cunningham. 1964. Transforming activities and base composition of deoxyribonucleases from strains of *Moraxella* and *Mima*. *J. Gen. Microbiol.* 37:353-367.
15. Henriksen, J., L. O. Frøholm, and K. Bøvre. 1972. Studies on bacterial surface translocation. 2. Correlation of twitching motility and fimbriation in colony variants of *Moraxella nonliquefaciens*, *M. bovis* and *M. kingii*. *Acta Pathol. Microbiol. Scand. Sect. B* 80:445-452.
16. Henriksen, S. D., and K. Bøvre. 1968. *Moraxella kingii* sp. nov., a haemolytic, saccharolytic species of the genus *Moraxella*. *J. Gen. Microbiol.* 51:377-385.
17. Henriksen, S. D., and K. Bøvre. 1968. The taxonomy of the genera *Moraxella* and *Neisseria*. *J. Gen. Microbiol.* 51:387-392.
18. Henriksen, S. D., and K. Bøvre. 1976. Transfer of *Moraxella kingae* Henriksen and Bøvre to the genus *Kingella* gen. nov. in the family *Neisseriaceae*. *Int. J. Syst. Bacteriol.* 26:447-450.
19. Jantzen, E., K. Bryn, T. Bergan, and K. Bøvre. 1974. Gas chromatography of bacterial whole cell methanolysates. V. Fatty acid composition of neisseriae and moraxellae. *Acta Pathol. Microbiol. Scand. Sect. B* 82:767-779.
20. Jantzen, E., K. Bryn, T. Bergan, and K. Bøvre. 1975. Gas chromatography of bacterial whole cell methanolysates. VII. Fatty acid composition of *Acinetobacter* in relation to the taxonomy of *Neisseriaceae*. *Acta Pathol. Microbiol. Scand. Sect. B* 83:569-580.
21. Jantzen, E., K. Bryn, and K. Bøvre. 1974. Gas chromatography of bacterial whole cell methanolysates. IV. A procedure for fractionation and identification of fatty acids and monosaccharides of cellular structures. *Acta Pathol. Microbiol. Scand. Sect. B* 82:753-766.
- 21a. Jantzen, E., K. Bryn, and K. Bøvre. 1976. Cellular monosaccharide patterns of *Neisseriaceae*. *Acta Pathol. Microbiol. Sect. B* 84:177-188.
22. Lautrop, H., K. Bøvre, and W. Frederiksen. 1970. A *Moraxella*-like microorganism isolated from the genito-urinary tract of man. *Acta Pathol. Microbiol. Scand. Sect. B* 78:255-256.
23. Snell, J. J. S., L. R. Hill, and S. P. Lapage. 1972. Identification and characterization of *Moraxella phenylpyruvica*. *J. Clin. Pathol.* 25:959-965.
24. Tatum, H. W., W. H. Ewing, and R. E. Weaver. 1974. Miscellaneous gram-negative bacteria, p. 270-294. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington D.C.