Moraxella kingii* sp.nov., a Haemolytic, Saccharolytic Species of the Genus *Moraxella

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SUMMARY

A new *Moraxella* species, *Moraxella kingii*, is described. It is characterized by β -haemolysis, acid production from glucose and maltose, and lack of catalase activity. The strain 4177/66, isolated from a nose swab, is designated as the type strain.

INTRODUCTION

In 1960 the late Miss Elizabeth O. King of Atlanta, Georgia, U.S.A., sent a few strains to one of us with a request for an opinion as to whether these were strains of the genus *Moraxella*, and, if so, whether they belonged to any well-defined species. Two of the strains were β -haemolytic and saccharolytic. Morphologically and in growth characteristics they showed considerable likeness to *Moraxella* species, but the conclusion was that they represented a new species, and that *Moraxella* appeared to be the most suitable genus to allocate it to. Later we obtained Miss King's permission to include these strains in studies on the transformation reactions of *Moraxella* and related organisms which were then in progress, but it was our understanding that Miss King would describe this new species as soon as she found time to do so. Unfortunately this was prevented by her death. When we isolated an additional strain of this species from a nose culture in 1966, we felt that publication of a description of the new species was desirable. Inquiries to the Communicable Disease Center in Atlanta revealed that there was no objection to the inclusion of Miss King's strains in our study.

METHODS

Organisms. One of the strains (4177/66) was isolated in Oslo in 1966. Two other strains of the material had previously been received from the late Miss Elizabeth O. King, and 6 strains, also from her collection, were received from Dr R. E. Weaver (Atlanta). The strains were designated as follows (with source in parentheses): 2941 (blood), 5530 (blood), 5963 (1) (blood), 8623 (blood), 9571 (throat), A 1702 (knee joint), A 2471 (bone lesion), A 6042 (throat) and 4177/66 (nose).

Morphological, cultural and biochemical tests. With some modifications these were identical with the procedures previously described for the examination of *Moraxella phenylpyrrovica* (Bøvre & Henriksen, 1967*b*). Haemolysis was studied on plates with ox, sheep, horse and rabbit blood in addition to the commonly used human blood agar medium. Tests for growth in citrate were made only in Koser's fluid medium.

Examination for acid production from carbohydrates was extended to the use of ascites agar slants with 1 % glucose, maltose, fructose, galactose, lactose, saccharose, arabinose, xylose, rhamnose, mannitol, dulcitol, sorbitol and glycerol, respectively.

DNA base determinations. Guanine + cytosine (G + C) contents of DNA were determined by Dr W. Szybalski (Professor of Oncology, University of Wisconsin) with the CsCl buoyant density method on extracts prepared in the authors' laboratory.

Transformation methods. A quantitative procedure with short-term DNA exposure and a more sensitive, but less accurate approach, with continuous DNA exposure, were employed. The principles of mutant selection, transformation techniques, transformant assay and control measures, have been extensively described previously (Bøvre, 1964, 1967*a, c*). The genetic marker used was exclusively one-step mutation to high streptomycin resistance.

RESULTS

Morphological, cultural and biochemical characteristics

Microscopical examination revealed coccoid to medium long diplo-bacteria with square ends, in pairs or chains. The bacteria were generally of rather small diameter as compared with previously studied *Moraxella* species. Pleomorphism was not pronounced in any of the strains. Microphotographs of two of the strains are given in Plate 1, fig. 1, 2. The organisms were Gram-negative, but in many instances some resistance to decolorization was observed. There were no distinct capsules. Motility was not observed.

All strains grew with small colonies, reaching diameters in the range 0.1–0.6 mm. in culture on human blood agar plates after 20 hr of incubation at 33° and 37°. Single colonies of some strains increased to 1–2 mm. in size on prolonged incubation. The colonies were generally of the low hemispherical type. In some instances there was a tendency towards colony growth down into the medium, with resulting pitting of the surface. In these cases the colonies were flat, extending very little from the surface of the surrounding medium, and the growth mass appeared adherent to the medium. The colonies were non-pigmented, translucent or slightly opaque, with a smooth and sometimes mucoid appearance. Their consistency varied from soft, giving easy emulsification in physiological saline (strain 5530), to coherent, like gonococci, with resulting spontaneous agglutination (the other 8 strains). The latter phenomenon was only temporary, since complete emulsification occurred after a while in saline.

In all strains a distinct, but most often very narrow zone of β -haemolysis was observed around colonies on human blood agar. The haemolysis was more pronounced on plates with ox, sheep, horse or rabbit blood.

There was no anaerobic growth. In semifluid nutrient agar stab cultures growth could be observed down to about 5 mm. below the surface. Very slight growth was observable with some of the strains at room temperature. There was no difference in growth capacity at 33° and 37°. Most strains survived exposure to 45° for 10 min., but not for 30 min. None of the strains resisted heating to 49° for 10 min.

Growth in peptone media such as the indole and nitrate media used was absent or very scarce, as it was on Triple Sugar Iron Agar (Difco) and in the MR–VP medium. Only very little improvement of growth was observed when 10 % (v/v) horse serum was added to fluid peptone media or when heated blood was substituted for ordinary blood in agar plates. This phenomenon seems to distinguish our strains from other

Table 1. Some cultural and biochemical characteristics of *Moraxella kingii*, as compared with other known species of the genus *Moraxella*

Species	Catalase production	Growth on Hugh and Leifson's medium	Growth in Koser's citrate medium	Nitrite production from nitrate	Urease activity	Oxidative deamination of phenylalanine and tryptophan	Serum liquefaction	Beta-haemolysis	Acid production from glucose
<i>M. lacunata</i> *	+	- or +	-	+	-	-	+	-	-
<i>M. nonliquefaciens</i>	+	-	-	+	-	-	-	-	-
<i>M. bovis</i>	+	+	-	-	-	-	+	+	-
<i>M. osloensis</i>	+	+	+	+	-	-	-	-	-
<i>M. phenylpyrrouvica</i>	+	+	+	+	-	-	-	-	-
<i>M. kingii</i>	-	-	- or (+)	+, (+) or - - or (+)	+	+	-	+	+ ‡

+ = positive reaction, (+) = doubtful reaction, - = negative reaction. † Weakly positive. ‡ Acid also from maltose.
* Including *M. liquefaciens*, as proposed by Henriksen & Bøvre (1968).

fastidious moraxellas, according to our experience. There was no satellite phenomenon with staphylococci. All strains were able to grow almost as well on high-quality nutrient agar as on the same medium with blood added.

The strains all had a remarkable inability to survive on blood agar plates at room temperature; cultures always became sterile in 6–12 days.

The catalase reaction was uniformly negative. This is in accordance with notes made by Miss Elizabeth O. King, placed at our disposal. The negative catalase reaction distinguishes our strains from all other known moraxellas, which have been extensively examined for this character in our laboratory (unpublished results). With the tetramethyl-*p*-phenylenediamine reagent all strains were strongly oxidase positive. With the dimethyl compound the reaction was also distinct with all strains, but perhaps weaker than with other known *Moraxella* species.

Strong acid production in 1 day was observed from glucose and maltose on ascites agar media. There was no apparent attack on the other carbohydrates and alcohols tested, except for very weak acid reactions in the galactose medium. No growth and no glucose attack was observed on Hugh and Leifson's medium. In 1% peptone liquid media with glucose growth was either absent or very weak, and only very late and irregular acid reactions were seen under these conditions.

No hydrogen sulphide or indole production was detected. Some results of cultural and biochemical tests are presented in Table 1, together with a comparison with other moraxellas recently studied in this laboratory. The results of antibiotic sensitivity tests are listed in Table 2.

DNA base composition

The guanine+cytosine (G+C) content of all DNAs examined (from strains 2941, 5530, A1702, 4177/66) was 44.5 moles %.

Table 2. *Sensitivity to antibiotics of the 9 strains of Moraxella kingii*

Antibiotic	Range of inhibition zones diam. (mm.)	Approximate range of minimum inhibitory concentrations (m.i.c.)*
Penicillin	37–54	0.02–< 0.005
Streptomycin	27–34	0.2–< 0.01
Chloramphenicol	36–45	0.1–< 0.03
Oxytetracycline	25–30	0.4–0.08
Erythromycin	31–45	0.3–< 0.008

* Method of Ericsson, Høgman & Wickman (1954). Minimal inhibitory concentration (m.i.c.) values calculated from zone diameters by means of regression equations for each antibiotic (Ericsson, 1960). M.i.c. given as i.u./ml. for penicillin, as µg./ml. for the other antibiotics.

Transformation reactions

The strain 4177/66 was simultaneously exposed to marker DNAs of the same strain and of the strains 2941, 5530 and A1702. There was a uniform distribution of interstrain transformation frequencies close to the frequency of intrastain (auto-logous) transformation, as presented in Table 3. The resulting ratios of inter- to intra-strain transformation can be seen to range from 4.6×10^{-1} to 9.2×10^{-1} . Values of this order are consistent with a close taxonomic relationship and similar to those previously found between strains of one and the same species (see Bøvre, 1967*b*).

In Table 4 are presented results of five quantitative transformation experiments designed to elucidate the transformation compatibility of *Moraxella kingii* with other *Moraxella* species. No such compatibility was observed. Depending on the sensitivity of the individual experiments (i.e. the transformability or state of competence of the

Table 3. *Quantitative streptomycin resistance transformation between strains of Moraxella kingii. Recipient strain 4177/66. Recipient count 7.6×10^7 /ml.*

Duration of DNA exposure 15 min. Donor mutants selected at 500 µg. streptomycin/ml.; transformants selected at 50 µg./ml.

Donor strain	Transformants per ml.	Ratio of inter- to intrastain transformation
2941	2.9×10^5	9.2×10^{-1}
5530	1.5×10^5	4.6×10^{-1}
A1702	2.2×10^5	6.9×10^{-1}
4177/66	3.2×10^5	—

Table 4. *Quantitative streptomycin resistance transformation in Moraxella, with Moraxella kingii as donor or recipient in interspecies experiments*

Recipient strain (recipient count/ml. in parentheses)	Donor strain	Transformants per ml.	Ratio of inter- to intrastain transformation
<i>M. nonliq</i> * 7784 (4.2×10^8)	<i>M. kingii</i> 5530, A1702, 4177/66	< 10	< 1.4×10^{-5}
	<i>M. nonliq.</i> 7784	7.0×10^5	
<i>M. osloensis</i> 5873 (9.0×10^8)	<i>M. kingii</i> 2941, 5530, A1702, 4177/66	< 10	< 8.3×10^{-6}
	<i>M. osloensis</i> 5873	1.2×10^6	
<i>M. bovis</i> ATCC 10900 (8.0×10^7)	<i>M. kingii</i> 2941, 5530, 4177/66	< 10	< 2.3×10^{-4}
	<i>M. bovis</i> ATCC 10900	4.4×10^4	
<i>M. lacunata</i> ATCC 11748 (3.2×10^7)	<i>M. kingii</i> 4177/66	< 10	< 3.7×10^{-3}
	<i>M. lacunata</i> ATCC 11748	2.7×10^3	
<i>M. kingii</i> 4177/66 (6.0×10^7)	<i>M. bovis</i> ATCC 10900	< 10	< 1.0×10^{-3}
	<i>M. phen.</i> † A390	< 10	< 1.0×10^{-3}
	<i>M. kingii</i> 4177/66	1.0×10^4	

Duration of DNA exposure 15 min. Donor mutants selected at 500 µg. of streptomycin per ml., transformants selected at 50 µg./ml.

* *nonliq.* = *nonliquefaciens*; † *phen.* = *phenylpyrovica*.

recipients in question) ratios of inter- to intra-strain transformation between *M. kingii* and the other species were below 1.4×10^{-5} for *M. liquefaciens*, below 8.3×10^{-6} for *M. osloensis*, below 2.3×10^{-4} for *M. bovis*, below 3.7×10^{-3} for *M. lacunata* and below 1.0×10^{-3} for *M. phenylpyrovica*. In all the experiments of Table 4, except for that with *M. lacunata* as recipient, the occurrence of transformants was also studied in continuous DNA exposure (without termination of DNA activity by DNase), which was performed in parallel. These experiments (not tabulated) were also negative, indicating that the transformation compatibility between *M. kingii* and the other species was considerably lower than the limits for these interactions listed above. Previous experiments with other organisms have shown that continuous DNA exposure was usually more than 100 times more sensitive for the detection of low transformation compatibility, as compared with the 15 min. DNA exposure technique (Bøvre, 1965).

In Table 5 are listed a few of the results obtained when highly competent *Moraxella* and *Neisseria* recipients were exposed continuously to DNAs of streptomycin-

resistant *Moraxella kingii* on the one hand, and simultaneously to other distantly related donors on the other. It can be seen that when the conditions of transformation permitted the *M. phenylpyrrovica* 2863 donor to give 246 transformants in the *M. osloensis* 5873 recipient, several donors of *M. kingii* were still completely unable to elicit such transformants. Since the compatibility between these strains of *M. phenylpyrrovica* and *M. osloensis* is known to be of the order 6×10^{-6} , as expressed by ratio of inter- to intra-strain transformation (Bøvre & Henriksen, 1967*b*), ratios of transformation between *M. kingii* and *M. osloensis* would be lower than 3×10^{-8} . Whereas *M. osloensis* shows some very distant relations to all other moraxellas (Bøvre, 1967*a*; Bøvre & Henriksen, 1967*a, b*), there was no indication of any transformation compatibility between the former species and *M. kingii*.

Table 5. *Semiquantitative comparison of low transformation frequencies (without termination of DNA exposure by DNase), with streptomycin resistance as the genetic marker*

Recipient strain	Donor strain	Transformants per plate	Approximate ratio of inter- to intrastrain transformation
<i>Moraxella osloensis</i> 5873	<i>M. phen.*</i> 2863	246	$6 \times 10^{-6} \ddagger$
	<i>M. kingii</i> 2941, 5530, A1702, 4177/66	0	$< 3 \times 10^{-8}$
	<i>M. nonliq.†</i> 7784	3500	$3 \times 10^{-5} \ddagger$
<i>Neisseria catarrhalis</i> NE 11	<i>M. osloensis</i> 5873	276	$2 \times 10^{-6} \ddagger$
	<i>M. phen.</i> 2863	613	$5 \times 10^{-6} \ddagger$
	<i>M. kingii</i> A1702	0	$< 9 \times 10^{-9}$
	<i>M. osloensis</i> 5873	1300	$3 \times 10^{-5} \ddagger$
<i>Neisseria ovis</i> 199/55	<i>M. phen.</i> 2863	561	$1 \times 10^{-5} \ddagger$
	<i>M. kingii</i> A1702	0	$< 3 \times 10^{-8}$

Donor mutants selected at 500 µg. of streptomycin per ml., transformants selected at 50 µg. per ml. For methodology, see Bøvre (1967*a*).

* *phen.* = *phenylpyrrovica*; † *nonliq.* = *nonliquefaciens*.

‡ Previously estimated (Bøvre, 1967*a*; Bøvre & Henriksen, 1967*b*).

Similar comparisons of low transformation frequencies in continuous DNA exposure were performed with *Neisseria catarrhalis* and *N. ovis* recipients (Table 5). These experiments revealed no transformants with *Moraxella kingii* donors under conditions where *M. nonliquefaciens*, *M. osloensis* and *M. phenylpyrrovica* donors elicited hundreds or thousands of transformants in the same recipients. The activities of the latter three donors had been estimated previously (Bøvre, 1967*a*; Bøvre & Henriksen, 1967*b*), and by means of these values the ratio of inter- to intra-strain transformation between *M. kingii* and *N. catarrhalis* would be situated below 9×10^{-9} , and between *M. kingii* and *N. ovis* the ratio would be below 3×10^{-8} . So far there is no indication of any transformation compatibility between *M. kingii* and *N. catarrhalis* or *N. ovis*, in contrast to the distinct, but often very distant relations in these terms previously found between all other moraxellas and these two *Neisseria* species.

Continuous DNA exposure experiments were also performed to detect compatibility between *Moraxella kingii* and *Neisseria flavescens* or *N. meningitidis*, with negative results. However, the recipients of these experiments were not of particularly high competence, so that a final decision cannot be made as regards absence of affinity between *M. kingii* and the latter *Neisseria* species.

DISCUSSION

The results indicate that the strains included in this study are a homogeneous group which is sufficiently distinct from all bacterial species known to us, to deserve the status of a species. This new species shares many of the characteristics of the genus *Moraxella* in morphology, cultural behaviour, oxidase reaction and antibiotic sensitivity, and it has a DNA base composition close to the range of the other *Moraxella* species. Like the majority of the latter it is transformable. On the other hand, the group shows marked differences from all the other known *Moraxella* species, the most conspicuous of which are the ability to produce acid from glucose and maltose, and the lack of catalase.

Flamm (1956) described a new species, *Moraxella saccharolytica*, which, like our organism, produced acid from glucose, maltose, fructose, saccharose, glycerol, starch; but not from arabinose, dulcitol, galactose, inositol, lactose, mannitol, melizitose, raffinose, rhamnose, salicin, sorbitol, xylose. It was non-haemolytic and it further differed from our strains in the following characters: good growth at 22°, viable on blood agar for 4 weeks, gelatin liquefaction, catalase production, and resistance to streptomycin, chloramphenicol and tetracyclines. As far as we know strains of this organism have not been maintained, at least no strain has been available to us; but it seems quite obvious that *M. saccharolytica* differs from our strains in too many characters to belong to the same species. The study of new isolates of *M. saccharolytica* would be necessary to determine the taxonomic position of this species and its relationships to other *Moraxella* species.

Whereas other *Moraxella* species show some, even if very slight, signs of mutual compatibility in transformation experiments, no sign of compatibility of the new species with other *Moraxella* species or with *Neisseria* could be detected. This suggests that the new species is more distantly related to the other *Moraxella* species than the latter are to one another. Nevertheless, we feel that the likeness to other species of the genus *Moraxella* is sufficient to admit the new species to this genus; we find at present no reasonable alternative.

The habitat of the new species is uncertain in view of the fact that so few strains are known; 3 of the 9 strains were isolated from throat or nose of patients. It may be pointed out that this organism could very easily be mistaken for α - or β -haemolytic streptococci or for haemolytic *Haemophilus* species, and it is possible that a systematic search for this organism might reveal that it is less rare than it may appear.

We name this species *Moraxella kingii* in honour of the late Miss Elizabeth O. King, who did the pioneer work on this species and who collected most of the existing strains. The strain 4177/66 is designated as type strain. It is being deposited in the National Collection of Type Cultures (Great Britain) and in the American Type Culture Collection. A condensed description of the species follows.

Description of Moraxella kingii sp. nov.

Micromorphology. Organisms coccoid to medium-sized rods, somewhat smaller than those of other *Moraxella* species, with square ends, in pairs and chains. Gram-negative, with some tendency to resist decolorization. Not capsulated. Non-motile. No endospores.

Colonies. Delicate, translucent or slightly opaque, 0.1–0.6 mm. in diameter after

20 hr on blood agar. Usually low hemispherical, smooth, but occasionally flat with some pitting of the agar surface, or of a mucoid appearance. No pigment. Consistency soft or coherent, in the latter case showing transient spontaneous agglutination in saline. Colonies surrounded by distinct zones of beta-haemolysis.

Relation to oxygen. Aerobe.

Temperature. Slight growth at room temperature (about 20°). Optimal growth at 33–37°. Usually killed by exposure to 45° for 10–30 min.

Growth requirements. Comparatively fastidious. Little or no growth in peptone media. No growth on Hugh & Leifson's medium. Practically no improvement of growth by addition of 10 % (v/v) horse serum to liquid peptone media. Growth on high quality nutrient agar almost as good as on blood agar. No apparent need for X or V factors.

Viability. Blood agar cultures become sterile within 6–12 days at room temperature.

Biochemical reactions. Catalase reaction negative. Oxidase reaction strongly positive with tetramethyl-, less strongly positive with dimethyl-*p*-phenylenediamine. Nitrate not reduced or slight reduction. Acid rapidly produced on ascites fluid agar slopes from glucose and maltose. Trace of acid from galactose. No acid from fructose, lactose, saccharose, arabinose, xylose, rhamnose, mannitol, dulcitol, sorbitol, glycerol. Gelatin or serum not liquefied. Indole or hydrogen sulphide not produced. Urease negative. No oxidative deamination of phenylalanine or tryptophan.

Antibiotic sensitivity. Highly sensitive to penicillin, streptomycin, chloramphenicol, oxytetracycline, erythromycin.

Habitat. Strains have been isolated from throat, nose, blood, bone lesion and joint.

Pathogenicity. Uncertain, probably low.

DNA base composition. 44.5 mole % guanine + cytosine (CsCl buoyant density method).

Genetic compatibilities. Strains are mutually compatible with ratios of interstrain to intrastrain transformant numbers close to 1. No compatibility detected with other *Moraxella* or with *Neisseria* species.

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Fig. 1

Fig. 2

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EXPLANATION OF PLATE

Fig. 1. *Moraxella kingii*, strain 4177/66 (type strain). Stained by Gram's method. × 1600.

Fig. 2. *Moraxella kingii*, strain A1702. Stained by Gram's method. × 1600.