Transfer of *Moraxella kingae* Henriksen and Bøvre to the Genus *Kingella* gen. nov. in the Family *Neisseriaceae*

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Many specialists have considered *Moraxella kingae* Henriksen and Bøvre not to belong to the genus *Moraxella* because it differs from the other *Moraxella* species in several characters and apparently does not have genetic affinities to the other species. For these reasons, the transfer of *M. kingae* to the genus *Kingella* gen. nov. in the family *Neisseriaceae* is proposed.

The species *Moraxella kingae* Henriksen and Bøvre, originally described under the name *M. kingii* (14), later corrected to *M. kingae* (8), was classified as a *Moraxella* species because it showed some striking similarities to members of *Moraxella*. Because of some likewise striking differences from *Moraxella* species, in particular production of acid from certain sugars and lack of catalase activity, this classification has not been well received and has not met with the approval of the Subcommittee on the Taxonomy of *Moraxella* and Allied Bacteria (19). Sufficient evidence is now available to justify the transfer of this species to a new, separate genus within the same family, *Neisseriaceae*. A summary of the evidence is given below.

Characters of *M. kingae* shared with moraxellae: (i) **micromorphology.** Plump, gram-negative rods and coccobacilli with obtuse or nearly square ends, occurring in pairs and short chains. Films from 18- to 20-h-old agar cultures show very distinct staining with some tendency to retain the Gram stain. No swimming motility. May or may not show twitching motility (12) and fimbriation with polar fimbriae (10, 11).

(ii) **Colonies.** Two different varieties may occur: (i) the spreading-corroding type of colony (13, 16), which is associated with twitching motility, fimbriation, and competence in transformation (6, 10, 12); and (ii) the smooth, stationary, normal type of colony, which does not show spreading, corrosion, twitching, fimbriation, or competence. Similar colony variations have been found in *M. nonliquefaciens* and *M. bovis* (5, 6, 12) as well as in *Neisseria gonorrhoeae* and *N. meningitidis* (11) and in *N. elongata* (to be published).

(iii) **Physiology.** Apparently physiologically fastidious, producing only moderate growth on many of the customary media, e.g., nutrient agar or peptone water media. Aerobic. Meso-

philic. Comparatively short-lived on blood agar at 35°C and at room temperature.

(iv) **Biochemical reactions.** Positive oxidase reaction, negative indole and H₂S reactions.

(v) **Antibiotic susceptibility.** Susceptible to penicillin, streptomycin, chloramphenicol, tetracyclines, erythromycin, and sulfonamides.

(vi) **Habitat.** Parasitic on mucous membranes. So far, isolated only from human sources.

Differences between *M. kingae* and moraxellae: (i) **biochemical reactions.** Acid production from glucose, maltose, and galactose (a trace). Negative catalase reaction, tested by dropping 10% H₂O₂ on to nutrient agar slant cultures. Hemolytic action on blood agar (in agreement with *M. bovis* and *N. ovis*, but in disagreement with other *Moraxella* species). (Opinions are divided as to whether the "false neisseriae" should be classified in *Branhamella* (9) or in *Moraxella* (15). We favor the latter alternative, but the designation *Neisseria* is used here while this taxonomic problem is reexamined experimentally.)

(ii) **Fatty acid composition.** All other *Moraxella* species cluster together with regard to fatty acid composition, whereas *M. kingae* has a clearly different fatty acid pattern as demonstrated in a phenogram, obtained by the unweighted pair group analysis of Sokal and Mitchner (17), of the fatty acid contents of *M. kingae* compared with other moraxellae and neisseriae.

Nondistinctive characters. In a number of other characters, there is variation among *Moraxella* species and no systematic difference between *M. kingae* and the other species.

Genetic evidence. Available genetic data showing affinities of the members of the family *Neisseriaceae* obtained by transformation experiments (2, 7), nucleic acid hybridization (3), and determination of the deoxyribonucleic acid

(DNA) base composition (4) may be roughly summarized as follows. (i) Commonly recognized *Moraxella* species (except *M. kingae*) appear to form a continuum of affinities, from very low (e.g., between *M. phenylpyruvica* and *M. nonliquefaciens*) to intermediate (e.g., between *M. bovis* and *N. ovis*) to high (as between the "classical moraxellae" *M. lacunata*, *M. nonliquefaciens*, and *M. bovis*) (2, 3, 7; unpublished data). (ii) The genetic affinities of *Acinetobacter* to this large group are generally very low, but sometimes they are distinct (as towards *N. catarrhalis*) and partly overlap with the lowest mutual affinities found in the oxidase-positive group mentioned (2, 3). (iii) The species of the "true" genus *Neisseria* (after exclusion of *N. catarrhalis*, *N. ovis*, and *N. caviae*) which have been tested appear mutually closely related and clearly separated from the above-mentioned organisms, with no distinct sign of affinity to them (2, 3). (iv) *M. kingae*, having been tested extensively in genetic transformation, has revealed no affinity to any strain of *Moraxella*, "false neisseriae," or *Acinetobacter*, and generally not to the genus *Neisseria*. However, an uncertain, marginal affinity was recently observed towards *N. elongata* (to be published).

DISCUSSION

The main differences in conventional characters, in fact the only significant ones, between *M. kingae* and the other *Moraxella* species are the acid production from sugars by *M. kingae*, a character it shares with many *Neisseria* species, and lack of catalase. Another phenotypic complex trait which clearly separates *M. kingae* from the other moraxellae and the "false neisseriae" is its pattern of fatty acid composition, as revealed by gas chromatography (17).

The genetic evidence also strongly indicates that *M. kingae* is not closely related to *Moraxella* or to other genera of the family *Neisseriaceae*. Even if significant, the very weak transformation interactions observed with *N. elongata* do not appear sufficient to consider *M. kingae* as having genus affinity to *Neisseria*. They may, however, indicate a relationship corresponding to different genera of the same family.

The rather unique fatty acid composition of *M. kingae* still falls within the general pattern of members of *Neisseriaceae*, with some similarity to that of members of the genus *Neisseria* (17).

The possibility of sufficient affinity of *M. kingae* to the neisseriae for the former to be included in *Neisseria* finds no support in the fol-

lowing observations. The guanine plus cytosine (G + C) content of the DNA of *M. kingae* when determined by buoyant density, 44.5 mol % (4), is significantly lower than that for the neisseriae. By gas chromatographic analysis of cellular monosaccharide content, *M. kingae* differs from all examined "true neisseriae" in its lack of heptose (E. Jantzen, K. Bryn, and K. Bøvre, Acta Pathol. Microbiol. Scand. Sect. B, in press). Finally, *M. kingae* reveals activities corresponding to the enzymes thymidine phosphorylase, nucleoside deoxyribosyltransferase, and thymidine kinase, which are absent from *Neisseria* species as well as from most *Moraxella* species (18).

Consequently, *M. kingae* appears isolated in the family *Neisseriaceae*. So also does the genus *Neisseria*, however, and there is no more reason to remove *M. kingae* from the family than to place *Neisseria* and *Moraxella* in different families. There are, rather, considerably indications (morphology like *Moraxella*, fermentation reactions like some *Neisseria* species, same habitat, G + C content within the range of *Neisseriaceae*, oxidase reaction, antibiotic susceptibility) that *M. kingae* does indeed fit into the family *Neisseriaceae*, and it would be difficult to find a more suitable family for this species. However, the lack of clearly demonstrable genetic homologies with the other members of the family and some of the phenotypic traits of *M. kingae* make it reasonable to create a new genus in the family *Neisseriaceae* for this species.

Only a few people have worked with *M. kingae*, and we feel that it is appropriate to derive the generic name for this and any related organisms from the name of Elizabeth O. King, the first to call attention to this organism. Consequently, we propose the name *Kingella* for the new genus (King, el'la. M.L. dim. ending -ella; M.L. fem.n. *Kingella*; named after Elizabeth O. King, an American bacteriologist).

The question arises whether there are any other candidates for the new genus. The possibility that the "new" moraxellas described by van Bijsterveld (1) and by Sutton et al. (20), which must belong to the same species on the basis of transformation data (unpublished), might be related to *M. kingae* has been suggested. However, there are some marked differences between these organisms and *M. kingae* in morphology, in attack on sugars, and in indole production as well as in DNA base composition. Previous (1, 4) and repeated experiments by buoyant density determinations by the method and calculation described (4) have given 44.5 to 45.0 mol% G + C for *M. kingae*

and 48 to 49 mol% for van Bijsterveld's strain, partly in simultaneous analytical centrifuge runs (unpublished data). The question of the relationship of these organisms must be left open for the time being. As far as we are aware, there are no other obvious candidates for this new genus.

Description of *Kingella* gen. nov.

The proposed genus *Kingella* is monospecific, and the following description therefore is a combined generic and specific description.

Micromorphology. The cells are coccoid to medium-sized rods, very much like those of *Moraxella* but slightly smaller, have square ends, and occur in pairs and short chains. Gram negative, with some tendency to resist decolorization. Not encapsulated. Not motile. Endospores are not produced.

Colonies. Two types of colonies occur. On blood agar, colonies of freshly isolated strains often first appear as small depressions, 0.1 to 0.5 mm in diameter, with a small central papilla. After incubation for 2, 3, or more days, the colonies show considerable peripheral spread, increasing to diameters of 4 to 5 mm. Thin granular zones of growth often surround the colonies. When the growth is scraped off, corrosion marks in the agar surface become apparent. This type of spreading-corroding growth is associated with polar fimbriation of the cells, twitching motility, competence in genetic transformation, and a tendency to pellicle formation when growing in fluid media. The second type of colony, which often arises in subcultures of the first type, is small, delicate, translucent or slightly opaque, 0.1 to 0.6 mm in diameter after 20 h on blood agar, low hemispherical, and smooth. After further incubation, the colonies increase somewhat in size, but do not spread or corrode, and are not associated with the same characters as the first type of colony. Both kinds of colony are surrounded by distinct zones of beta-hemolysis. The consistency is soft or coherent. No pigment.

Relation to oxygen. Aerobic.

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

Growth requirements. Relatively fastidious. Little or no growth occurs in peptone media. There is no growth on Hugh and Leifson O/F medium and practically no improvement of growth by the addition of 10% (vol/vol) horse serum to liquid peptone media. Growth on high quality nutrient agar is almost as good as that

on blood agar. There is no apparent need for X or V factors or for CO₂ in the atmosphere.

Viability. Blood agar cultures become sterile within 6 to 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 slightly reduced. 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, or glycerol. Gelatin and serum are not liquefied. Indole and hydrogen sulfide are not produced. Urease negative. No oxidative deamination of phenylalanine or tryptophane.

Antibiotic susceptibility. Highly susceptible to penicillin, streptomycin, chloramphenicol, oxytetracycline, and erythromycin.

Habitat. Parasitic on human mucous membranes. Strains have been isolated from throat, nose, blood, bone lesions and joints.

Pathogenicity. Uncertain, probably low.

DNA base composition. 44.5 to 45 mol% G + C (CsCl buoyant density method).

Genetic compatibilities. Strains are mutually compatible with ratios of interstrain to intrastrain transformant numbers close to 1. No compatibility with moraxellae or neisseriae except for an uncertain, marginal affinity to *N. elongata*.

Type strain. (For original designation, see reference 16.) 4177/66 (= ATCC 23330 = NCTC 10529).

REPRINT REQUESTS

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LITERATURE CITED

1. Bijsterveld, O. P. van. 1970. New *Moraxella* strain isolated from angular conjunctivitis. *Appl. Microbiol.* 20:405-408.
2. Bøvre, K. 1967. Transformation and DNA base composition in taxonomy, with special reference to recent studies in *Moraxella* and *Neisseria*. *Acta Pathol. Microbiol. Scand.* 69:123-144.
3. Bøvre, K. 1970. Pulse RNA-DNA hybridization between rod-shaped and coccial species of the *Moraxella-Neisseria* group. *Acta Pathol. Microbiol. Scand. Sect. B* 78:565-574.
4. Bøvre, K., M. Fianndt, and W. Szybalski. 1969. DNA base composition of *Neisseria*, *Moraxella* and *Acinetobacter* determined by measurement of buoyant density in CsCl gradients. *Can. J. Microbiol.* 15:335-338.
5. Bøvre, K., and L. O. Frøholm. 1972. Variation of colony morphology reflecting fimbriation of *Moraxella bovis* and two reference strains of *M. nonliquefaciens*. *Acta Pathol. Microbiol. Scand. Sect. B* 80:639-640.
6. 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.
7. Bøvre, K., J. E. Fuglesang, N. Hagen, E. Jantzen, and L. O. Frøholm. 1976. *Moraxella atlantae* sp. nov. and its distinction from *Moraxella phenylpyruvica*. Int. J. Syst. Bacteriol. 26:511-521.
 8. 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.
 9. Catlin, B. W. 1970. Transfer of the organism named *Neisseria catarrhalis* to *Branhamella* gen. nov. Int. J. Syst. Bacteriol. 20:155-159.
 10. Frøholm, L. O., and K. Bøvre. 1972. Fimbriation associated with the spreading-corroding colony type of *Moraxella kingii*. Acta Pathol. Microbiol. Scand. Sect. B 80:641-648.
 11. Henriksen, J. 1972. Bacterial surface translocation: a survey and a classification. Bacteriol. Rev. 36:478-503.
 12. 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.
 13. Henriksen, S. D. 1969. Corroding bacteria from the respiratory tract. I. *Moraxella kingii*. Acta Pathol. Microbiol. Scand. 75:85-90.
 14. Henriksen, S. D., and K. Bøvre. 1968. *Moraxella kingii* spec. nov., a haemolytic, saccharolytic species of the genus *Moraxella*. J. Gen. Microbiol. 51:377-385.
 15. Henriksen, S. D., and K. Bøvre. 1968. The taxonomy of the genera *Moraxella* and *Neisseria*. J. Gen. Microbiol. 51:387-392.
 16. Henriksen, S. D., and K. Bøvre. 1969. Corroding and spreading colonies in *Moraxella nonliquefaciens*. Acta Pathol. Microbiol. Scand. 76:85-90.
 17. 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.
 18. Jyssum, S., and K. Bøvre. 1974. Search for thymidine phosphorylase and thymine kinase in *Moraxella*, *Acinetobacter*, and allied bacteria. Acta Pathol. Microbiol. Scand. Sect. B 82:57-66.
 19. Lessel, E. F. 1970. International Committee on Nomenclature of Bacteria, Subcommittee on the Taxonomy of *Moraxella* and Allied Bacteria. Minutes of Meeting, 11 August 1970. Int. J. Syst. Bacteriol. 21:213-214.
 20. Sutton, R. G. M., M. G. O'Keefe, M. A. Bundock, J. Jeboult, and M. Tester. 1972. Isolation of a new *Moraxella* from a corneal abscess. J. Med. Microbiol. 5:148-150.