Neisseria elongata subsp. *glycolytica* subsp. nov.

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A rod-shaped, saccharolytic strain of *Neisseria* isolated from the throat of a patient with pharyngitis and considered to represent a new subspecies of *Neisseria elongata* is described. The strain differs from *N. elongata* subsp. *elongata* in producing acid from glucose, in giving a strong catalase reaction, and in the consistency of colonies on agar media. The name *N. elongata* subsp. *glycolytica* is proposed for the new subspecies, of which strain 6171/75 (= ATCC 29315 = NCTC 11050) is the type.

The purpose of this paper is to name and describe a rod-shaped strain of *Neisseria* isolated from the human pharynx and identified as belonging to a new subspecies of *Neisseria elongata*.

MATERIALS AND METHODS

Bacterial strains. Strain 6171/75 was isolated from the throat of a 22-year-old woman suffering from acute pharyngitis. A culture of a nose swab from this patient was sterile. The throat culture did not contain known pathogens, but it did contain a mixture of bacteria thought to be commensals, including a strain designated as 6171/75.

In addition to strain 6171/75, *N. meningitidis* M1 (12), *N. elongata* M2, *N. catarrhalis* Ne 11, and *Moraxella nonliquefaciens* 4663/62 were used in this study.

Methods. The methods used were mainly those described previously by Bøvre and Henriksen (5).

Transformation of streptomycin resistance was performed as described by Lie (13). Deoxyribonucleic acid (DNA) was prepared by the method of Bøvre (2). The DNA solution was treated with ribonuclease and Pronase before the final deproteinization. During transformation, log-phase cells were exposed to DNA (10 µg/ml) for 30 min and were then treated with deoxyribonuclease and plated after proper dilution.

Antibiotic susceptibility tests were carried out by means of filter-paper disks manufactured by A/B Biodisk, Stockholm, according to the directions given by the manufacturer.

RESULTS

Description of strain 6171/75: *Micromorphology.* Very small, short, slender rods with a marked tendency to occur in pairs; gram negative; nonmotile; very similar to the cells of *N. elongata* (4, 6).

Colonies. In primary culture on 5% human blood agar, the colonies were relatively large (2 to 3 mm in diameter after about 20 h of incubation), gray, opaque, moderately raised with a flat top, and smooth with a soft, homogeneous consistency. The colonies were easily emulsi-

fied in saline. There was no hemolysis on human blood agar. After a few days, the colonies appeared to have a slight yellowish tinge on 5% human blood agar.

In the first subculture (Fig. 1) on the same medium, as well as in later subcultures, but primarily in the first few transfers, four different colony types appeared. Type 1 was a relatively large, rather flat colony, which after 3 to 4 days developed a slightly irregular surface and margin (Fig. 2). Under the colony, there was distinct "corrosion" (Fig. 3) with a narrow furrow corresponding to the margin of the colony and a small central pit (9). After about 1 week, tiny papillae often appeared on top of these colonies. Type 2 was a round, convex, nearly hemispherical smooth colony with a shiny surface, about 1 to 2 mm in diameter after 24 h (Fig. 4). When the colonies were gently removed, a barely visible mark was seen of the agar surface, but there was no "corrosion." Type 3 was a colony similar to the second type, but with low, tongue-like processes from one or both sides or from around the margin of the colony (Fig. 1). Subcultures of this type of colony produced mixtures similar to those seen in the first subculture. Type 4 was a flat, smooth colony with a slightly irregular margin (Fig. 5) that was later derived from the type 2 colony.

Subcultures of types 1, 2, and 4 tended to become more stable in subculture, but there still remained a tendency to variation, particularly of type 1 towards type 2.

We looked for signs of surface spreading of the colonies (as seen in the spreading-corroding [SC] type of colony of certain *Moraxella* species [3, 8, 10] and in *N. elongata* [4]) in numerous cultures incubated in a humid atmosphere for 1 week or more but, with one exception, with negative results. The exception was a blood-agar culture of colony type 1, where thin, granular zones closely resembling those seen around colonies of some *Moraxella* species (8, 10) and of

FIG. 1. Blood agar, 48 h $\times$ 4. One large irregular colony (type 1), many round smooth colonies (type 2), several smooth colonies with offshoots. From 1st subculture of strain 6171/75. Magnification, $\times$ 4.

FIG. 2. Blood agar, 4 days. Large colony with irregular margin (type 1). Magnification, $\times$ 4.

N. elongata (4) appeared around some of the colonies (Fig. 6), mostly colonies growing close to a contaminating *Staphylococcus* or *Micrococcus* colony. A stimulating effect of contaminating colonies on this kind of surface spread has previously been noted in other spreading bacteria (11). Similar zones have so far not been observed in cultures of colony types 2 and 4.

Physiology. The strain was a strict aerobe. Growth occurred at 30°C (poor), but not at 20°C, was only slightly improved by a humid atmos-

phere, and was not further improved by the addition of CO₂ to the atmosphere. The strain stayed viable for at least 10 days at 35°C in a humid atmosphere. It appeared to be somewhat fastidious and showed rather poor growth in most fluid media but reasonably good growth on nutrient agar slants.

Biochemical reactions. A weakly acid reaction, due to oxidation, was produced from glucose in the course of several days. No acid was produced from galactose, fructose, xylose, rhamnose, mannose, maltose, sucrose, or lac-

FIG. 3. Blood agar, 4 days. Corrosion marks on agar surface after removal of type 1 colonies. Magnification, $\times$ 4.

FIG. 4. Blood agar, 4 days. Round, smooth colonies (type 2). Magnification $\times$ 4.

FIG. 5. Blood agar, 4 days. Large, flat, noncorroding colony derived from type 2 (colony type 4). Magnification $\times 4$.

FIG. 6. Blood agar, 8 days. Colonies with thin, granular, spreading zones surrounding large colony. Magnification, $\times 5$.

tose. The oxidase reaction was positive with dimethyl- and tetramethyl-*p*-phenylenediamine. The catalase reaction, carried out on an agar slant culture, was very strongly positive. Nitrate was not reduced, but nitrite was reduced without the appearance of gas in the Durham tube. The H_2S reaction was weakly positive by the lead acetate paper-strip method. The following tests were negative: indole, citrate, phenylalanine and tryptophane deami-

nase, and liquefaction of gelatin and coagulated serum.

Poly- β -hydroxybutyrate inclusions were not found in cells grown in Hutner medium (5) with β -hydroxybutyrate or in ordinary media.

Antibiotic susceptibility. The strain was strongly susceptible to penicillin, streptomycin, chloramphenicol, tetracyclines, and erythromycin.

Transformation of streptomycin resistance. Strain 6171/75 was transformed nearly as efficiently by DNA from *N. elongata* as by autologous DNA, and vice versa (Table 1). Cross-transformation with *N. meningitidis* showed that the heterologous transformation both ways produced 23% of the number of transformants produced by autologous DNA. Strain 6171/75 was not transformed by DNA from *N. catarrhalis* and *M. nonliquefaciens*, nor were the latter two species transformed by DNA from strain 6171/75.

DISCUSSION

The physiological and biochemical reactions of strain 6171/75 suggest that it belongs to the genus *Neisseria*. This is supported by the results of transformation experiments, which reveal extremely close affinities between this strain and *N. elongata* strain M2 and strong compatibilities also with *N. meningitidis*.

Morphologically, and in most other characters, the strain showed very marked resemblance to *N. elongata*, so close that one might think that the strain represents a biotype of that species. The difference between the two, however (acid production from glucose, the catalase reaction, which is negative in most strains of *N. elongata* [1, 4] and strongly positive in strain 6171/75, and the very marked differences in the consistency of the colonies: clay-like, coherent, and hydrophobic in *N. elongata*; and soft, homogenous, and easily emulsifiable in strain 6171/75) are more striking than the differences between some of the named species of *Neisseria*. If similar principles were to be followed in the classification of this strain as have been used previously in this genus, we feel that the strain would represent a new species. However, in view of the very close genetic relationship between this strain and *N. elongata*, we classify it as a subspecies of *N. elongata*. We propose the name *Neisseria elongata* subsp. *glycolytica* (Gr. *n. gleukos* sweet wine; Gr. adj. *lyticus* dissolving; M. L. adj. *glycolyticus* glucose-splitting) for this new subspecies. The type strain, 6171/75, has been deposited in the National Collection of Type Cultures, Colindale, London (accession number, NCTC

TABLE 1. Transformation of streptomycin resistance between strains 6171/75, *N. elongata* M2, and *N. meningitidis* M1

Recipient strain (colony count/ml)	Donor strain	Transformants/ml	Ratio of interstrain to autologous transformation
Strain 6171/75 (3.3×10^9)	<i>N. elongata</i> M2	1.4×10^5	5.0×10^{-1}
	<i>N. meningitidis</i> M1	9.1×10^3	3.3×10^{-2}
	Strain 6171/75	2.8×10^5	
<i>N. elongata</i> M2 (9.4×10^7)	Strain 6171/75	1.7×10^4	3.9×10^{-1}
	<i>N. meningitidis</i> M1	3.4×10^2	7.7×10^{-3}
	<i>N. elongata</i> M2	4.4×10^4	
<i>N. meningitidis</i> M1 (4.7×10^6)	Strain 6171/75	5.9×10^4	1.8×10^{-2}
	<i>N. elongata</i> M2	4.4×10^4	1.4×10^{-2}
	<i>N. meningitidis</i> M1	3.2×10^6	

11050) and in the American Type Culture Collection, Rockville, Md. (accession number, ATCC 29315).

The subspecies appears to be a missing piece in the "*Neisseria* puzzle." The known species include saccharolytic and asaccharolytic cocci and an asaccharolytic rod-shaped species, and the existence of a saccharolytic rod-shaped organism is not surprising.

The pattern of colony variation in this strain resembles that found in several *Moraxella* and *Neisseria* species. Thus, the type 1 colony of this strain may be analogous to the SC type of colony of *Moraxella* species (3) or to the type 1 colony of *N. gonorrhoeae*, and the type 2 colony may be analogous to the normal (N) colony type of *Moraxella*.

Objections may be raised against the establishment of a new subspecies based on only a single strain, but previous experience with *N. elongata* indicates that this may not always be inadvisable. The establishment of that species was on the basis of a single strain, but it was very quickly confirmed by several investigators (1, 4, 7), and it may be anticipated that the description of this strain will incite others to report similar findings.

Further studies of this strain comparing it with other members of the family *Neisseriaceae* are in progress, and they will be published elsewhere.

REPRINT REQUESTS

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