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Analyses of Deoxyribonucleic Acid of *Neisseria caviae* and other *Neisseria*

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ABSTRACT

LAMACCHIA, ELIZABETH H. (University of Maryland, College Park), AND MICHAEL J. PELCZAR, JR. Analyses of deoxyribonucleic acid of *Neisseria caviae* and other *Neisseria*. J. Bacteriol. **91**:514–516. 1966.—The base composition of deoxyribonucleate preparations extracted from 11 strains of *Neisseria caviae*, expressed in terms of mole per cent guanine plus cytosine ranged from 47.7 to 50.4, with an average of 49.2. This compared closely with the values obtained for DNA from two strains of *N. perflava*, which were 49.2 and 50.5%, as well as with published values for most other *Neisseria* species. The values obtained for five strains of *N. catarrhalis*, however, ranged from 42.3 to 45.7%. These results suggest that *N. caviae* may be more closely related to *Neisseria* spp. other than *N. catarrhalis*.

The phenotypic characteristics of organisms are dependent on the deoxyribonucleic acid (DNA) and its composition, so it is reasonable to assume that those strains of bacteria with nearly identical properties would have similar DNA compositions. Investigations have shown that those strains which closely resemble each other phenotypically have nearly identical mole per cent guanine plus cytosine (as an expression of the DNA base composition), and that those species and genera which resemble other species and genera have similar ranges of mole per cent base composition (8). However, it is recognized that it is the sequential arrangement of the bases in DNA which is significant in terms of comparisons for similarity, i.e., identical mole per cent guanine plus cytosine does not in itself establish that two strains are identical. Nevertheless, differences in the mole per cent of these bases would indicate differences between the strains.

Studies by Catlin and Cunningham (3, 4) with *Neisseria* species revealed similar base compositions of DNA for *N. meningitidis*, *N. perflava*, *N. subflava*, *N. flava*, *N. sicca*, and *N. flavescens*: 49 to 51 mole per cent guanine plus cytosine. However, they also found that the mole per cent of guanine plus cytosine in *N. catarrhalis* DNA ranged between 40 and 44.

A species of *Neisseria* which has not been studied in this respect is *N. caviae* (10, 11), isolated from the pharyngeal region of guinea pigs at the University of Maryland. It was described as gram-negative paired cocci with adjacent sides flattened. The colonies [incubated for 48 hr on Trypticase Soy Agar (BBL) at 35 C] were 2 mm in diameter, circular, entire, and convex; they had smooth glistening surfaces, a butyrous consistency, and produced a brown water-soluble pigment.

The purpose of this investigation was to establish the relationships of the base composition of *N. caviae* to *N. catarrhalis* and other *Neisseria* species.

MATERIALS AND METHODS

Cultures. All strains of *N. caviae*, *N. catarrhalis*, and *N. perflava* were maintained on half-strength dextrose-starch agar at 35 C and were transferred at monthly intervals. All cultures were examined for their morphological and cultural characteristics, as well as for the following biochemical activities: glucose, levulose, sucrose, and maltose fermentations; oxidase reaction; and catalase production.

DNA extraction. The cells were grown in Trypticase Soy Broth (BBL) plus 0.3% yeast extract on a rotary shaker. Approximately 5 to 10 g of wet-packed cells harvested from this medium were washed in saline-ethylenediaminetetraacetate solution and were subsequently lysed by the action of sodium lauryl sulfate (6). The DNA was extracted by the method of Catlin (2) and deproteinized by the method of Kay, Simmons, and Dounce (5). Bovine pancreatic ribonuclease was added in approximately 0.001 mg/ml of DNA suspension, and the mixture was incubated at 37 C for 30 min to remove contaminating ribonucleic acid. All final DNA preparations were maintained in sterile standard saline citrate (6) at 4 C.

DNA analyses. Base composition determinations

were performed chromatographically according to the method described by Bendich (1). An amount of 5 to 10 mg of the DNA preparation was lyophilized in a glass-stoppered test tube. When the sample was dry, 0.1 to 0.2 ml of 70% perchloric acid (0.1 ml per 5 mg of DNA) was added, and the mixture was heated at 100 C for 60 min with occasional agitation (9). The mixture was diluted 1:1 with distilled water, ground to produce a uniform suspension with the residue, and centrifuged to remove the residue.

The clear supernatant fluid was placed directly on Whatman no. 1 filter paper in the form of a line. Identical lines of 2 N perchloric acid were also placed on the paper to serve as blanks. After the lines dried, the chromatograph paper was placed in a descending chromatograph chamber with concentrated hydrochloric-isopropanol solution [16.7 ml of concentrated hydrochloric acid, 65.0 ml of isopropanol, and water to 100 ml (12)]. The solvent front was allowed to run for 15 to 16 hr.

The paper was dried upside down in a hood with concentrated ammonia in beakers at the bottom of the paper. Better definition of the guanine spot was achieved by exposing the paper briefly to ammonia vapor, since guanine was on the paper in the form of the hydrochloride (1). The chromatogram was examined in a dark room over an ultraviolet light. The separated bases, i.e., guanine, adenine, cytosine, and thymine, could be seen as dark patches against a background of general paper fluorescence. There was no evidence for the presence of uracil.

Each spot was eluted with 5 ml of 0.1 N hydrochloric acid. The eluates were decanted into small test tubes and left standing overnight to permit the small paper shreds to settle. The corresponding areas in the blank lanes on the chromatograph were treated in the same manner (12).

The eluates were read in matched silica cells on a Beckman DB spectrophotometer against the blank eluates. The optical density of each base was measured at the maximal absorption wavelength in the ultraviolet range. Quantitative determinations of the bases were obtained by the absorption maxima extinction technique, and the base composition of the DNA was expressed as the mole per cent guanine plus cytosine.

RESULTS

All of the *Neisseria* strains used in this study were catalase- and oxidase-positive. *N. caviae* and *N. catarrhalis* strains did not ferment any of the carbohydrates used, whereas all strains of *N. perflava* fermented glucose, levulose, sucrose, and maltose.

The base composition of the *Neisseria* studied, expressed in terms of mole per cent guanine plus cytosine, is shown in Table 1. These values represent averages of several replicate determinations. The mole per cent guanine plus cytosine in the DNA samples of two strains of *N. perflava* was 49.2 and 50.5. These values are in agreement with previously reported determinations (3, 7). The base composition of the *N. catarrhalis* strains ranged from 42.3% to 45.7%. These values are slightly higher than those reported by Catlin and Cunningham (4). However, they did find one strain, *N. catarrhalis* NCTC 4103, which had a mole per cent guanine plus cytosine averaging 44.5, compared with the value of 45.7 obtained in this investigation. This difference (1.2%) may well result from differences of techniques between the laboratories.

TABLE 1. *Mole per cent guanine plus cytosine content of DNA preparations from Neisseria*

Neisseria species	Mole per cent guanine plus cytosine
N. perflava 0799	50.5
N. perflava N7	49.2
N. catarrhalis NCTC 4103	45.7
N. catarrhalis 7269	43.4
N. catarrhalis 1 VI A	43.4
N. catarrhalis 6666	42.6
N. catarrhalis ATCC 8176	42.3
N. caviae GP-8	50.4
N. caviae J	50.2
N. caviae N	50.2
N. caviae GP-3	50.1
N. caviae F	49.6
N. caviae GP-4	49.3
N. caviae GP-13	48.6
N. caviae GP-11	48.4
N. caviae GP-16	48.4
N. caviae Q	48.4
N. caviae R	47.7

The *N. caviae* strains were characterized by a 47.7% to 50.4% range of guanine plus cytosine content. These values compare closely with those obtained with the *N. perflava* strains, both of which are much higher than the values obtained for the *N. catarrhalis* strains. All of the *N. caviae* strains studied were nearly identical in their base composition. Thus, from the standpoint of base composition, *N. caviae* compares closely with the *Neisseria* species other than *N. catarrhalis*.

Experiments are in progress to determine the transformation compatibilities among these species of *Neisseria*, particularly *N. caviae* and *N. catarrhalis*.

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