Morphology and Nucleic Acid Composition of the *Azotobacter* Bacteriophages

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Long-tailed azotophages of two different serological groups have deoxyribonucleic acid species whose densities correspond to a molar percentage of guanine plus cytosine of 52 to 53. Similar measurements on the short-tailed phages gave values of 56 to 57 moles percent. The azotophages include representatives of the three morphological types classified by Bradley as groups A, B, and C.

Bacteriophages specific for some strains of the genus *Azotobacter* have been isolated by this laboratory (2, 3) and classified into serological groups on the basis of cross-neutralization of the phages in heterologous antisera. This study deals with azotophage morphology and nucleic acid composition. The results are correlated with the original serological groupings described by Duff and Wyss (2) and the morphological groupings suggested by Bradley (1).

A. vinelandii, strain O, was used for the propagation and titrating of the azotophages (2). Phage identity was confirmed by phage neutralization by its homologous antiserum. For electron microscopy, the azotophages were negatively stained with 2% sodium phosphomolybdate (pH 8.0), 2% sodium phosphotungstate (pH 6.0), or 2% uranyl acetate (pH 4.3). Negatively stained preparations were observed with an Hitachi HS-7S electron microscope. Concentration and purification for deoxyribonucleic acid (DNA) extraction were done by the method of Reilly and Spizizen (4). The buoyant density of azotophage DNA was determined in an analytical centrifuge by using a cesium chloride equilibrium density gradient. *Bacillus subtilis* phage 2-C DNA was added to calibrate each CsCl gradient. The buoyant density of the azotophage DNA was calculated and converted by the method of Schildkraut, Marmur, and Doty (5) to molar percentage of the bases contributed by the guanine plus cytosine (GC) fraction.

The azotophages from the serological groups studied can be separated into three different morphological types (J. J. Konvicka, L. Pope, and O. Wyss, *Bacteriol. Proc.*, p. 195, 1969) i.e., Bradley's groups A, B, C. Group A is represented

by phages A-11 and A-14. Figure 1a shows A-14 with its tail sheath in the extended form. In Fig. 1b, the tail sheath is contracted and reveals the core. Phages A-13 (Fig. 4) and A-31 (Fig. 5) with long noncontractile tails are group B types. Group C includes azotophages A-12 (Fig. 2a, b, and c), A-21, A-22 (Fig. 3), and A-24. This group is characterized by possession of a short noncontractile tail. A-12 (Fig. 2a, b, and c) demonstrates tail configurations which appear as a tail plate with several projections (Fig. 2b) or a plate with a single projection (Fig. 2c). Figure 3 shows several A-22 phages which appear to have short fibrillar tails.

Serological grouping, morphological data, DNA density, and moles percent GC are presented in Table 1. The two morphological groups characterized by a long tail (groups A and B) have similar DNA densities. Two serological groups are represented. Members of serological group 20 (A-21, A-22, A-23, and A-24) are morphologically identical, and the buoyant densities of their DNA species are similar. The morphology and the DNA buoyant density of phage A-12 are characteristic of the 20 series.

The serological groupings of azotophages isolated by Duff and Wyss (2) could not be correlated with morphology. The fraction of DNA bases consisting of GC could not be correlated with the morphological types of Bradley but showed two distinct groupings, the long-tailed phages with 52 to 53% GC and the short-tailed phages with 56 to 57% GC.

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FIG. 1-5. Negative stains of azotophages *A-12*, *A-13*, *A-14*, *A-22*, and *A-31*.

FIG. 1. (a) *A-14*: 2% sodium phosphomolybdate, pH 8.0. Bar indicates 0.05 μm . (b) *A-14*: 2% phosphotungstic acid, pH 6.0. Bar indicates 0.1 μm .

FIG. 2. (a) *A-12*: 2% uranyl acetate, pH 4.3. Bar indicates 0.1 μm . (b) *A-12*: 2% uranyl acetate, pH 4.3. Bar indicates 0.05 μm . (c) *A-12*: 2% uranyl acetate, pH 4.3. Bar indicates 0.05 μm .

FIG. 3. *A-22*: 2% uranyl acetate, pH 4.3. Bar indicates 0.05 μm .

FIG. 4. *A-13*: 2% uranyl acetate, pH 4.3. Bar indicates 0.05 μm .

FIG. 5. *A-31*: 2% uranyl acetate, pH 4.3. Bar indicates 0.05 μm .

TABLE 1. *Composite of azotophage morphology and deoxyribonucleic acid (DNA) analysis*

Group	Description	Azotophages ^a	DNA density (g/cm ³)	Guanine plus cytosine (moles %)
A	Long contractile tail Head diam = 120nm; tail diam = 20nm; tail length = 180 nm	A-11	1.712	53.1
		A-14	1.712	53.1
B	Long noncontractile tail Head diam = 60nm; tail diam = 10nm; tail length = 170 nm	A-13	1.711	52.0
		A-31	1.711	52.0
C	Short noncontractile tail Head diam = 60nm	A-12	1.716	57.1
		A-21	1.716	57.1
		A-22	1.716	57.1
		A-23	1.715	56.1
		A-24	1.715	57.1

^a Serological groupings consist of the 10 series (A-11 to A-14), the 20 series, and the 30 series. A-12 serology has changed from the original isolate.

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