Construction of a Restriction Map of the *Gluconobacter* Bacteriophage A-1 Genome

By N. K. ROBAKIS,¹* N. J. PALLERONI,² M. BOUBLIK³ AND
C. W. DESPREAUX²

¹ New York State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, ¹ NY 10314, USA

² Department of Microbiology, Hoffmann-La Roche Inc., Nutley, NJ 07110, USA

³ Roche Institute of Molecular Biology, Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ 07110, USA

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The molecular weight of *Gluconobacter* bacteriophage A-1 DNA was determined by restriction analysis and electron microscopic measurements to be about 30×10^6 . The DNA has cohesive ends and forms ring-like monomers. A circular map with the relative positions of the cleavage sites for the restriction enzymes *Eco*RI, *Sma*I, *Xho*I and *Bam*HI is proposed.

INTRODUCTION

Members of the genus *Gluconobacter* carry out limited oxidations of alcohols and sugars resulting in high yields of industrially important products (Ward, 1967; Asai, 1968). In spite of the economic importance of these transformations, little is known at present about the physiology and genetics of the organisms involved. The isolation of the first bacteriophage specific for members of this genus (phage A-1) was reported recently (Schocher *et al.*, 1979), as a result of studies of abnormalities found in the microbiological process for the oxidation of D-sorbitol to L-sorbose by *Gluconobacter oxydans*. Since then the isolation and characterization of two more *Gluconobacter* bacteriophages has been reported (Robakis *et al.*, 1985). This communication describes the results of molecular characterization of the phage A-1 genome.

METHODS

Culture conditions. Strain J of *Gluconobacter oxydans* was obtained from the culture collection of Hoffmann-La Roche, Nutley, NJ, USA. The strain was grown in mannitol medium (Robakis *et al.*, 1985). Bacteriophage A-1 was propagated on the bacterial strain using mannitol agar medium and was purified as described in the preceding paper (Robakis *et al.*, 1985).

Isolation of phage DNA. SDS, EDTA (pH 8.0) and proteinase K (Boehringer) were added to the phage A-1 suspension to give final concentrations of 0.5% (w/v), 20 mM and 50 µg ml⁻¹ respectively. The mixture was incubated (1 h) at 65 °C and then extracted with an equal volume of phenol equilibrated with 100 mM-Tris/HCl buffer, pH 8.0. This step was followed by extractions with phenol/chloroform (1:1), and with chloroform. Finally, the DNA was dialysed against 10 mM-Tris/HCl, pH 8.0, 1 mM-EDTA, and stored at 4 °C (Maniatis *et al.*, 1982).

Restriction analysis. The phage DNA was digested with various restriction enzymes (New England Biolabs) following the manufacturer's instructions. Double digestions were done by first incubating 5 µg of DNA with one enzyme, followed by treatment of samples (1 µg) of the digest with the second enzyme. The DNA fragments were separated by gel electrophoresis on 1% (w/v) agarose gels, using a horizontal apparatus (22 × 13.5 cm). The electrophoresis buffer, staining and visualization of the DNA bands are described in the preceding paper (Robakis *et al.*, 1985). DNA fragments were isolated from the gels by electro-elution into dialysis bags. The eluted DNA was purified by passage through Elutip columns (Schleicher & Schuell, Keene, NH, USA) according to the manufacturer's directions, and by ethanol precipitation.

RESULTS AND DISCUSSION

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Fig. 1. Partial restriction map of phage A-1 DNA. Lengths of fragments are given in kb.

the 11 kb long *Sma*I fragment and subsequent digestion with *Eco*RI resulted in three fragments 6.4, 3 and 1.5 kb long; (b) isolation of the 18 kb *Eco*RI fragment and digestion with *Sma*I gave two fragments 11.5 and 6.4 kb long; (c) isolation of the large *Eco*RI fragment and digestion with *Bam*HI give three fragments 18, 6.4 and 2.5 kb long (data not shown).

Electron microscopy. The length of the fully extended A-1 DNA molecule, as determined by conventional electron microscopy, is $13.8 \mu\text{m} \pm 5\%$ (mean of 50 molecules). This value corresponds to a molecular weight of 28×10^6 , which is in reasonable agreement with the molecular weight of about 30×10^6 estimated from the results of the restriction analysis. We have been unable to detect any circular molecules in heated (75 °C for 5 min) or non-heated specimens. Possibly, most of the cohesive hydrogen bonded ends pulled apart during preparation for electron microscopy, as described for λ DNA (Thomas & Davis, 1975) and for the actinophages SH10, SH11 and SH12 DNA (Klaus *et al.*, 1979).

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