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Analysis of DNA of Phage M1 of *Rhizobium meliloti*

Analiza DNA faga M1 *Rhizobium meliloti*

Rhizobiophages occur commonly in the rhizosphere of legumes and are often associated with susceptible rhizobia (4, 5, 7, 11, 14, 15). Rhizobiophages are of interest because of their usefulness in genetic analysis of *Rhizobia*, their role in selective elimination of native rhizobia and their use as markers in ecological studies using phage-sensitive strains.

Biological characteristic of phages of all major groups of *Rhizobium* have been reported (5, 10, 11, 15, 18), but literature pertaining to characteristic of DNA of rhizobiophages is limited (1, 9).

MATERIALS AND METHODS

Bacterial strains and phages. As a host for phage M1 (11) was used strain L5.30 of *R. meliloti* (6). For multiplication of phage λcI857, nutrient broth LB was employed (16).

DNA isolation. Phage M1 was propagated on the *R. meliloti* strain L5.30 using the double agar layer technique (2). The phage was pelleted by means of twice repeated ultracentrifugations at 28,000 r.p.m. for 1h. Phage λcI857 was harvested upon the UV induction of *E. coli* M5107 at 42°C. Phage DNAs were isolated using the method described by Maniatis et al. (13).

DNA base composition. The average G + C content of the DNA of phage M1 was measured by using the thermal denaturation method described by Mandela and Marmur (12). The thermal melting point of DNA was determined in the UNICAM SP500 spectrophotometer.

Filling recessed 3'ends of double-stranded DNA. Klenow fragment of *E. coli* DNA polymerase I and terminal deoxynucleotidyl transferase, in the presence of Co^{++} as cofactor, were used to label recessed 3'termini of DNA of phage M1 and λcI857 according to methods described by Maniatis et al. (13).

RESULTS AND DISCUSSION

In previous studies we have indicated that phage M1 of *R. meliloti* strain L5.30 belongs to the *Siphoviridae* morphological group of phages (11). The main feature of these phages is long, noncontractile tail (17). The DNA of phage M1 is double-stranded particle sensitive to many restriction enzymes (11). Using Klenow fragment of *E. coli* DNA polymerase I and terminal transferase, in the presence of Co^{++} as cofactor, we have labeled recessed 3' termini of DNA of phage M1 and λI857 (Fig. 1). This experiment has indicated that DNA of phage M1 of *R. meliloti*, similar as the DNA of phage λ*c*I857, has linear configuration with protruding 5' termini.

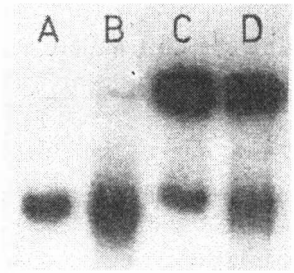

Fig. 1. Labeling with [α–^{32}P] d ATP of recessed 3' ends of double-stranded DNA: phage M1 (A, C) and phage λ*c*I857 (B, D) using terminal transferase in the presence of Co^{++} (A, B), Klenow fragment of *E. coli* DNA polymerase I (C, D)

The base composition of phage M1 DNA was determined by simple spetroscopic method on the basis of thermal melting point (T_m = 74°C) using the equation:

$$G + C = 2.44 \quad (T_m - 53.9) \text{ mol\%}.$$

The content of G + C of phage M1 DNA was calculated to be 48.8 mol%, which falls within the ranges of G + C (50–62 mol%) that have been reported for other rhizobiophages (1, 9).

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STRESZCZENIE

Badano strukturę i skład zasad G + C w DNA łagodnego faga M1 *R. meliloti*. Stwierdzono, że DNA tego faga jest cząsteczką liniową, dwuniciową z wystającymi końcami 5'. Na podstawie temperatury topnienia DNA (T_m) wykazano również, że zawartość zasad G + C w kwasie dezoksyrybonukleinowym faga M1 wynosi 48,8 mol%.