

STUDIES ON THE GROWTH OF THE PHAGE T4 IN CELL DIVISION MUTANTS DV1211 AND AD651 OF *Escherichia coli* AT NORMAL AND HIGH TEMPERATURES

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ABSTRACT

The *E. coli* cell division mutants DV1211 and AD651 show a reduction in the rate of phage T4 adsorption to the cell surface. The growth of phage T4 is normal in these mutants and delay of T4 production at 42°C seems due to impaired phage maturation mechanisms.

INTRODUCTION

Masamune (1975) reported a thermosensitive cell division mutant of *E. coli* C600, mutant strain ts612, that does not support the multiplication of phage T4 and lambda at 42°C, although it supported the growth of phage T7, after 60 minutes of incubation.

Cell division mutants in *E. coli* are conditional temperature sensitive mutants with deficient septum formation after DNA replication. When the incubation temperature is shifted from 30°C to 42°C, cell division is immediately interrupted.

Mutants DV1211 and AD651 are thermosensitive cell division mutants of *E. coli* C600 (Câmara, 1977). Similarly to the ts612 mutant (Masamune, 1975), their cell division is interrupted immediately at 42°C, but DNA synthesis is not and some membrane proteins show alterations at 42°C. Mutations ts612, DV1211 and AD651

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are mapped near 75 (Masamune, 1975), 0 (Santos, 1975) and 3.5 minutes (Câmara, 1977), respectively, in the *E. coli* K12 genetic map (Bachmann *et al.*, 1976).

In this paper we study the growth of phage T4 in the mutants DV1211 and AD651 and in the parental strains C600 and C620.

MATERIAL AND METHODS

Strains: The following *E. coli* strains were used: *E. coli* C600, also used to prepare and titrate phage T4; *E. coli* AD651 and DV1211, cell division mutants obtained by mutagenesis of *E. coli* C620; and *E. coli* C620, strain C600 lysogenic for phage lambda. The phage T4 used in this work was the wild type strain, received from three sources: Dr. K.B. Low, Dr. A.A.C. Leitão, and Carnegie Institute's phage collection. All these phages behave in the same way.

Media: YET broth contained 8 g of tryptone (Difco), 5 g of yeast extract (Difco) and 5 g of sodium chloride (Difco) per liter. YET plates and agar top were prepared by the addition of 15 and 8 g of agar (Difco) respectively. The phages lysates and titrations were performed in YET broth.

One step growth experiments: They were realized according to Eisenstark (1967), so that we could measure the latent period (the minimum time between the adsorption of phage to host cell and the lysis of the host cell with release of phage progeny) and the burst-size (the mean yield of phage particles per infected bacterium). The experiments were conducted in cultures grown under aeration at 37° and 42°C.

Rates of phage adsorption: They were determined according to Eisenstark (1967) and defined as the adsorption rate of infective phage particles on bacterial cells, in 10 minutes, in a multiplicity of infection of 0.1.

Electron microscopy: Bacterial cells at 42°C after 60 minutes of infection and uninfected controls were fixed with glutaraldehyde, centrifuged and prepared for observation in electron microscopy according to Dunley *et al.* (1973).

RESULTS

The burst-sizes of phage T4 for *E. coli* strains C600, C620, DV1211 and AD651 at 37° and 42°C are shown in Table I. The burst-sizes at 37°C for all the strains were about 134 phages/cell and at 42°C their burst-sizes decreased about 10 times, when measured at the same time as the burst-size at 37°C (50-60 minutes).

Table I - Burst-sizes (BS) of phage T4 at 37° and 42°C after 60 minutes of infection¹.

Strain	BS at 37°C	BS at 42°C
C600	137	10
C620	132	15
DV1211	127	13
AD651	140	20

1. Each value is the average of 5 experiments.

The rates of phage adsorption were decreased about two to three times in the strains DV1211 and AD651 when compared to the parental strains C600 and C620 (Table II). Although adsorption constant is a first order reaction, that is, not affected by temperature, we tested it at 42°C to detect any possible conformational change in bacterial cell envelope induced by temperature shift as occur for example, in some cell mutants (Ferreira, 1982).

Table II - Rate of phage adsorption (K) in cells of *E. coli* strains (in cm³ min⁻¹).

Strain	K at 30°C	K at 42°C
C600	1.5×10^{-9}	2.0×10^{-9}
C620	1.0×10^{-9}	1.0×10^{-9}
DV1211	7.0×10^{-10}	6.3×10^{-10}
AD651	4.6×10^{-10}	4.6×10^{-10}

Cultures of C600, C620, DV1211 and AD651 strains infected with phage T4 show complete lysis measured by optical density in about 90 minutes at 37°C and in about 240 minutes at 42°C. Therefore the lysis was delayed at 42°C. On the other hand, the production of infective phages in cultures at 37°C is complete in 50 minutes, while this time increases to 150-180 minutes at 42°C (see Table III). In both conditions lysis stops at 2×10^{10} infective phages/ml, approximately. Although lysis was delayed at 42°C, we observed mature virus particles inside bacterial cells at 42°C after 60 minutes of infection (Figure 1), but their infectivity, when liberated from cells with chloroform treatment, were low.

Table.III - Data concerning infection with the phage T4 in the strains C600, C620, DV1211 and AD651 at 37° and 42°C.

Item	37°C	42°C
% of phages that inject DNA into the bacterial cell at 10 minutes	> 80	> 80
Latent period (minutes)	~20	~20
Burst-size at 60 minutes (phages/cell)	~134	~10
Period (minutes) of final phage production ($\sim 10^{10}$ phages/ml) in culture corresponding to the normal burst-size	~50	150-180
Period of complete lysis in culture measured by decrease of optical density (minutes)*	~ 90	~ 240

* For these experiments we started with 2×10^8 bacterial cells/ml of aerated culture infected at a multiplicity of infection of one phage to ten bacteria. For phage titration, samples of cultures were previously treated with chloroform.

Finally, when cultures at 42°C were transferred to 37°C after 10, 15 and 20 minutes of T4 infection, the burst-size was normal and occurred at the same time as expected at 37°C. On the other hand, when the inverse procedure was realized the T4 growth was the same as observed at 42°C, as previously described.

DISCUSSION

Cell division mutants DV1211 and AD651 supported the growth of phage T4 at 37°C; for that characteristic they differ from the ts612 mutant (Masamune, 1975). Contrary to this author we observed that growth of T4 in *E. coli* C600 and its strains is decreased at 42°C. This is probably not related to the *E. coli* genetic background. At 42°C, complete lysis occurs in a period of time longer than that required at 37°C. At 42°C mature phage particles can be found in the cell at the time in which lysis would occur at 37°C. The delayed phage yield at 42°C points out a probable thermic sensibility of the phage maturation. Nevertheless, the results of temperature shift infected culture experiments during the eclipse period suggest that DNA and protein synthesis are not affected at 42°C.

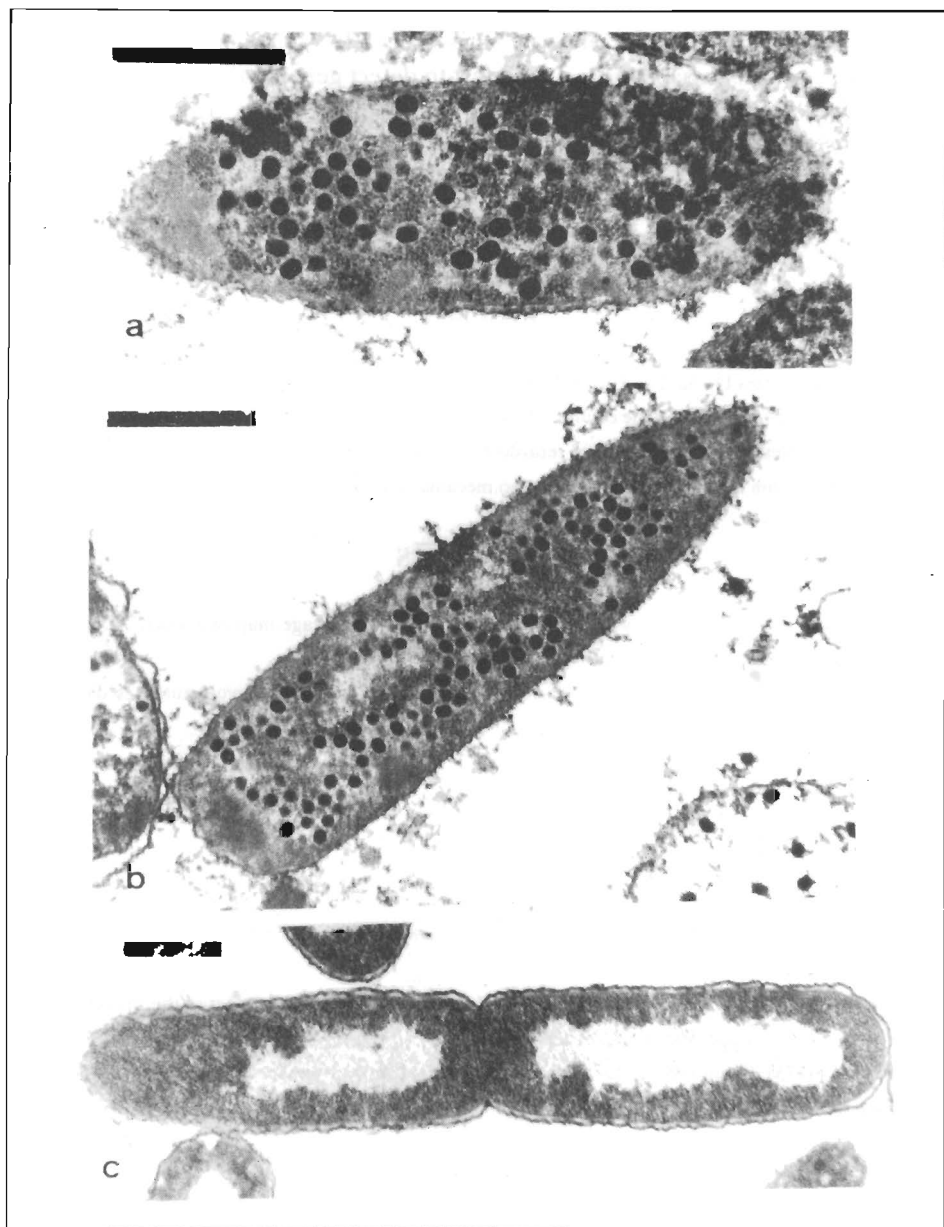


Figure 1 - Electron microscopy of *E. coli* at 42°C after 60 minutes of infection with the phage T4: (a) DV1211 mutant; (b) AD651 mutant; (c) non-infected control. Photos of strains C600 and C620 were similar (the bars correspond to 0.5 μ).

Mutants DV1211 and AD651 show some permanent alterations in the cell surface, demonstrated by the rate of adsorption decrease for phage T4. The facility and accuracy of this parameter is a good test to detect general alterations in the cell surface of bacterial mutants.

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RESUMO

Os mutantes DV1211 and AD651 da divisão celular em *E. coli* apresentam redução na taxa de adsorção do fago T4 à sua superfície celular. O crescimento deste fago nos referidos mutantes é normal, porém, a 42°C apresenta um considerável retardo na sua liberação por lise celular. O fenômeno parece estar associado à uma alteração termoinduzida no mecanismo de maturação do fago T4.

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