SHORT COMMUNICATION

Influence of Prophage on the Efficiency of Plating of *Escherichia coli* K12

By ANGELA TARNAPOLSKY-LEWKOWICZ,[1] DAVID DORIGO[2] AND DARCY F. DE ALMEIDA[1]*

[1] *Laboratório de Fisiologia Celular, Instituto de Biofísica, UFRJ, Centro de Ciências da Saúde, Bld. G, 21941 Rio de Janeiro, RJ, Brasil*

[2] *Instituto de Matemática, UFRJ, Centro de Ciências Matemáticas e da Natureza, 21941 Rio de Janeiro, RJ, Brasil*

(*Received 22 December 1980*)

The efficiency of plating of *Escherichia coli* K12 and its derivatives K12 C600 and K12 C600(λ) is diminished during the transition period from the lag to the exponential growth phase in cultures grown in tryptone broth. The phenomenon is suppressed in cultures of the strain K12 C600(λind). The reduction in the efficiency of plating is transitory and not influenced by temperature between 30 and 42 °C.

INTRODUCTION

The integration of a temperate phage genome in a host bacterium chromosome often results in alterations of host functions. Examples of this kind occurring in the *Escherichia coli–λ* phage system are (i) the increased fitness of a lysogen compared with the non-lysogenic isogenic strain (Edlin *et al.*, 1975), (ii) the dependence of the bacterial synchronization ability on the presence of a lambdoid prophage (Cox & Strack, 1972; Wolosker & Almeida, 1977), and (iii) a temporal delay in the transition from the lag to the exponential growth phase in liquid cultures of lysogenic strains (Strack & Cox, 1971). We have re-examined the latter phenomenon and found a diminished efficiency of plating (e.o.p.) among various *E. coli* K12 strains during the transition period between the lag and exponential phase, which is not observed when the bacteria are lysogenized with a non-inducible mutant prophage (λind).

METHODS

Organisms. The *Escherichia coli* strains used were K12 C600 (F^- *thr leu thi supE lac ton* λ^-), K12 C600(λ) and K12 C600(λind) (both isogenic with C600 but lysogenic for λ and λind, respectively), and strain B (prototrophic).

Medium. Bacteria were grown in tryptone broth (TB). For plating purposes, 1·5% (w/v) agar was added to this medium. Cultures were started as 1 : 100 dilutions of overnight cultures in TB medium.

Sampling. Samples were taken every 15 min, from the time of inoculation of stationary phase cells up to the exponential growth phase, for at least 135 min of culture; appropriate dilutions were plated on TB plates and incubated overnight. Plating was performed in triplicate. Experiments were carried out at 30, 37 or 42 °C. Between 6 and 12 experiments were performed with each strain, under each specified experimental condition. Strain B was used as a control.

Statistical analysis. Counts of viable cells, or colony-forming units (c.f.u.), obtained as described above, were plotted against time. The best fit was determined using the least square method, goodness of fit being expressed by the coefficient R^2. The F test was used to determine the significance of the slope at the 5% level.

0022-1287/81/0000-9706 $02.00 © 1981 SGM

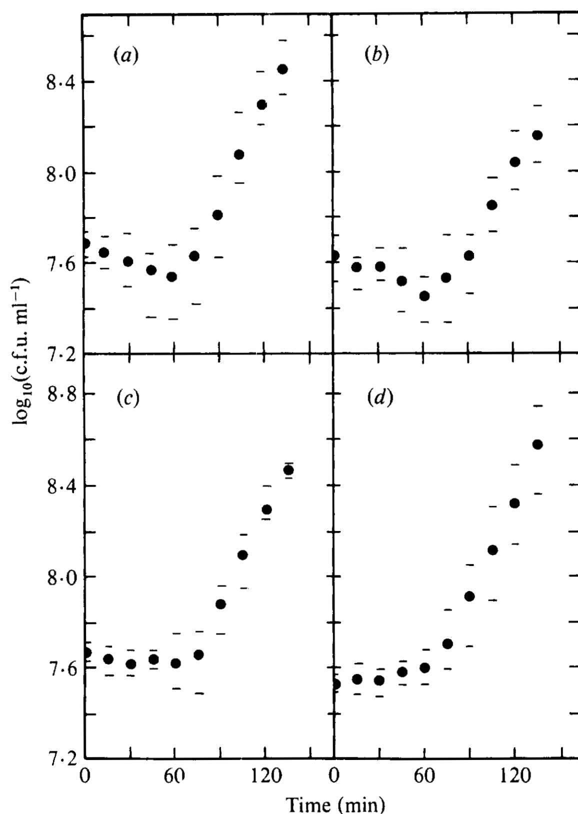

Fig. 1. Growth curves of *Escherichia coli* strains in TB medium at 37 °C. Points are average values of 6 to 12 experiments (see Table 1), each point being determined in triplicate in every experiment. Horizontal bars indicate the range of values observed. Strains: (*a*) K12 C600; (*b*) K12 C600(λ); (*c*) K12 C600(λind); (*d*) B.

Table 1. *Analysis of the variation in viable cell concentration of growth curves shown in Fig. 1*

For this analysis the linear equation $Y = \beta_0 + \beta_1 t + \varepsilon$ was used, where $Y = \log N_t$; N_t is the viable cell concentration at time t; $\beta_0 = \log N_0$; N_0 is the viable cell concentration at zero time; β_1 is the growth constant; $\varepsilon = \log \varepsilon'$; and ε' is the experimental error.

E. coli strain	No. of experiments	Coefficient of determination, R^2 (%)	$\hat{\beta}_0$	$\hat{\beta}_1 \pm S(\hat{\beta}_1)$
K12 C600	12	96·88	17·68	−0·0050 ± 0·0009
K12 C600(λ)	9	92·86	17·57	−0·0064 ± 0·0015
K12 C600(λind)	6	58·06	17·18	−0·0016 ± 0·0009
B	6	89·19	17·35	+0·0022 ± 0·0009

RESULTS AND DISCUSSION

Figure 1 shows the variation, as a function of time, of the viable cell count in cultures of the various *E. coli* strains grown at 37 °C. The statistical analysis of these data is summarized in Table 1. For strain K12 C600, a significant decrease was observed in the concentration of viable cells (expressed as c.f.u. ml^{-1}) during the transition from the lag phase to the exponential growth phase; lysogenization with phage λ did not alter the pattern of decreased viable cell concentration during this transition (Fig. 1*a*, *b*). However, cultures of strain C600 made lysogenic with the mutant prophage λind exhibited a growth curve indistinguishable

from that of the control strain *E. coli* B (Fig. 1*c*, *d*, and Table 1). Similar results to those shown in Fig. 1 and Table 1 for growth at 37 °C were obtained at both 30 and 42 °C.

It is thus not the fact of a strain being lysogenic that brings about the decreased e.o.p. during the growth phase transition; under certain circumstances, such as the presence of a mutant non-inducible prophage in the host chromosome, the reverse is true. Mutant *λind* is characterized by its insensitivity to the usual prophage-inducing agents, such as ultraviolet radiation or X-rays (Jacob & Campbell, 1959), due to the production of altered repressor molecules. It thus seems that not only the repressor synthesis but also the quality of the molecule being synthesized influences some domains of the host cell physiology.

This work has been supported by funds from CEPG/UFRJ, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq grant PIG-II/1354) and Financiadora de Estudos e Projetos (FINEP grants 375/CT and B-76-79-074). We thank Celso Pereira for technical assistance. A.T.-L. acknowledges receipt of a CNPq scholarship. D.F.A. is a CNPq research fellow.

REFERENCES

Cox, J. H. & Strack, H. B. (1972). Synchronous growth of *Escherichia coli* C600 dependent on a lambdoid prophage. *Virology* **50**, 912–915.

Edlin, G., Lin, L. & Kudrna, R. (1975). λ lysogens of *E. coli* reproduce more rapidly than non-lysogens. *Nature, London* **255**, 735–737.

Jacob, F. & Campbell, A. (1959). Sur le système de répression assurant l'immunité chez les bactéries lysogènes. *Comptes rendus hebdomadaire des séances de l'Académie des Sciences* **248**, 3219–3221.

Strack, H. B. & Cox, J. H. (1971). Lambdoid prophage modifying the kinetics of host cell division. *Virology* **43**, 719–721.

Wolosker, H. B. M. & Almeida, D. F. de (1977). Timing of cold-sensitive stages in the cell division cycle of *Escherichia coli* K12. *Journal of General Microbiology* **99**, 99–105.