Genet. Res., Camb. (1966), **7**, *pp.* 277–279
Printed in Great Britain

On the localization of a factor responsible for host-controlled restriction in *Escherichia coli* K(P1)

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(*Received* 18 *January* 1966)

INTRODUCTION

Recent experiments indicate that an essential step in the restriction of phage λ.C by *E. coli* K(P1) bacteria may involve an enzyme located on the surface of the cells (Schell & Glover, 1966*a*, *b*), and a surface-localized nuclease has been implicated in the restriction of non-glucosylated T4 DNA by *E. coli* B (Fukasawa, 1964; Molholt & Fraser, 1965). Neu & Heppel (1964*a*, *b*) have shown that several enzymes, alkaline phosphatase, latent RNase, 5′-nucleotidase, acid phosphatase, cyclic phosphodiesterase and an RNA inhibited DNase can be partly or completely released by *E. coli* cells during spheroplast formation. These authors also reported that surface-localized enzymes can also be released by EDTA treatment followed by washing at 4°. The results reported in this paper show that the restriction of phage λ.C by K(P1) cells is markedly reduced when the cells are treated with EDTA and washed several times with cold distilled water.

MATERIALS AND METHODS

Bacterial strains and bacteriophages: (see Schell & Glover, 1966*a*).

Media: Tryptone-broth (Arber & Dussoix, 1962). λ adsorption buffer: 0·01 M tris HCl and 0·01 M $MgSO_4$.

Methods: The general phage techniques are as described by Adams (1950). Special techniques relating to λ are those described by Arber (1958, 1960). EDTA cold-wash treatment was carried out by the method of Neu & Heppel (1964*b*).

RESULTS AND DISCUSSION

Table 1 shows the efficiency of plating (e.o.p.) of λ.C on K(P1) after EDTA treatment followed by washing with cold distilled water. EDTA alone has little or no effect but after three successive cold water washes the e.o.p. increases by a factor of 10^4. When 20% sucrose was omitted from the EDTA medium no significant effect on restriction was observed. Similar experiments have shown that an EDTA cold-wash treatment increases the e.o.p. of λ.C and λ.B on *E. coli* K and increases the transmission of T3 in K(P1) (Schell, unpublished results).

It appears from these experiments that a factor essential for restriction can be washed away from cells after EDTA treatment and this factor is located on the cell surface. Neu & Heppel (1964*b*) have shown that from 70 to 100% of the following enzymes can be removed by EDTA treatment followed by a single cold wash: alkaline phosphatase, cyclic

phosphodiesterase, 5′-nucleotidase and acid phosphatase. An RNA-inhibited DNase and a latent RNase were only slightly affected by this procedure, less than 10% being removed, although these enzymes had also been shown to be surface localized (Neu & Heppel, 1964*a*).

Previous results (Schell & Glover, 1966*b*) led us to postulate that a surface-localized DNase played an essential role in the restriction process. It is therefore relevant that, when the cold-wash treatment is applied only once, as was the case in Neu and Heppel's experiments, only a small effect on restriction is observed (experiment 3, Table 1), whereas repeated washings showed an increasing effect (experiment 4, Table 1). This

Table 1. *The effect of EDTA cold-wash treatment on the restriction of λ.C by* E. coli *K(P1)*

Experiment number	Treatment	Number of infective centres on K(P1)	Adsorption (%)	e.o.p. of infective centres
1	None	$2{\cdot}5 \times 10^{2}$	96·0	$5{\cdot}0 \times 10^{-7}$
2	EDTA	$5{\cdot}6 \times 10^{1}$	90·5	$1{\cdot}25 \times 10^{-7}$
3	EDTA and one cold wash	$1{\cdot}1 \times 10^{3}$	90·0	$2{\cdot}5 \times 10^{-6}$
4	EDTA and three cold washes	$1{\cdot}0 \times 10^{6}$	90·0	$2{\cdot}0 \times 10^{-3}$

1. *Untreated cells.* K(P1) bacteria were grown in tryptone broth, harvested and washed three times in 0·01 M tris HCl at pH 8·0 and finally resuspended in 0·03 M tris HCl at pH 8·0 plus 20% sucrose and held at 24° for 60 min. Restriction was measured by resuspending the cells in λ adsorption buffer at 5×10^{9} cells per ml., challenging with 5×10^{8} λ.C at a multiplicity of 0·1, adsorbing for 15 min. followed by anti-λ serum treatment for 5 min. at 37°. The number of infective centres was assayed on K(P1) indicator bacteria.

2. *EDTA treatment.* K(P1) bacteria were grown in tryptone broth, harvested and washed three times in 0·01 M tris HCl at pH 8.0 and finally resuspended in 0·03 M tris HCl at pH 8.0 plus 20% sucrose and 10^{-4} M EDTA and held at 24° for 10 min. Restriction was measured as in (1) above.

3. *EDTA and one cold-wash treatment.* As in (2) above, finally the treated cells were resuspended in cold distilled water at 1×10^{10} bacteria per ml. and shaken on a rotary shaker at 4° for 15 min. Restriction was measured as in (1) above.

4. *EDTA and three cold-wash treatments.* As in (3) above, repeating the cold water wash three times.

result could be explained if the surface-located RNA-inhibited DNase was more firmly bound to the cytoplasmic membrane than the other enzymes that are readily released by the cold-wash treatment. Recently Cordonnier & Bernardi (1965) have shown that endonuclease I has a surface location in *E. coli* but that it is released more slowly than other surface-localized enzymes. Preliminary experiments have shown that partially purified cytoplasmic membrane fractions, isolated from K and K(P1) bacteria, possess DNase activity but this activity was without specificity towards DNAs of different host-modifications (Schell & Glover, unpublished results).

Previously we have shown that ability of K(P1) bacteria to restrict the growth of λ.C can be reduced by heat treatment and by growth to saturation in yeast-extract–phosphate–glucose medium (Schell & Glover, 1966*a*, *b*). In both these cases it was possible to restore in a large measure the restricting capacity of the cells by resuspending them in hypertonic media.

The results in Table 2 however show that when K(P1) bacteria are subjected to EDTA treatment followed by three cold washes and finally resuspended in hypertonic media there was only a slight restoration of their restricting ability. This indicates that the EDTA cold-wash treatment actually removes a factor essential for restriction and that in this case osmotic shock does not restore restriction.

Table 2. *The effect of hypertonic media on the restriction of λ.C by K(P1) bacteria after EDTA cold-wash treatment*

Experiment number	Adsorption medium	Number of infective centres on K(P1)	Adsorption (%)	e.o.p. of infective centres
1	λ adsorption buffer	$2{\cdot}4 \times 10^5$	98	5×10^{-4}
2	λ adsorption buffer + 1 M NaCl	$4{\cdot}7 \times 10^5$	66	$1{\cdot}4 \times 10^{-3}$
3	λ adsorption buffer + 2M glucose	$3{\cdot}4 \times 10^5$	68	1×10^{-3}

K(P1) bacteria were grown in tryptone broth, treated with EDTA followed by three cold water washes (see Table 1, experiment 3) and resuspended in the adsorption media indicated. Restriction was measured as in Table 1.

SUMMARY

The restriction of phage λ.C by K(P1) cells is reduced when the cells are subjected to an EDTA cold-wash treatment which has been shown to remove surface-localized enzymes. We conclude that a surface-localized enzyme plays an essential role in host-controlled restriction.

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