SHORT COMMUNICATION

Restriction Enzyme Analysis of *Bacillus subtilis* Bacteriophage ϕ 105 DNA

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The recognition sites on ϕ 105 DNA for the restriction endonucleases *EcoRI*, *BglII*, *SmaI*, *KpnI*, *SstI*, *SalI*, *XhoI*, *NcoI*, *PstI*, *HindIII*, *ClaI*, *EcoRV* and *MluI* have been mapped. The sites for *EcoRI* are shown to be different from those published earlier. The DNA from ϕ 105 contains no recognition sites for the endonucleases *BamHI* and *XbaI*.

INTRODUCTION

The *Bacillus subtilis* temperate bacteriophage ϕ 105 (Birdsell *et al.*, 1969) and its deletion mutant ϕ 105DI:1t (Flock, 1977) have been widely used recently as vectors for cloning vegetative and sporulation genes of *B. subtilis* (Iijima *et al.*, 1980; Jenkinson & Mandelstam, 1983; Savva & Mandelstam, 1984; J. Errington, unpublished). However, phage ϕ 105 DNA has been analysed with only a few restriction endonucleases (Scher *et al.*, 1978; Perkins *et al.*, 1978; Jenkinson & Mandelstam, 1983). The restriction endonuclease *EcoRI* cleaves phage ϕ 105 DNA into eight fragments which have been lettered B to I and placed in a linear order by Perkins *et al.* (1978). This *EcoRI* map has been used as a reference for the ordering of restriction fragments generated by *KpnI* (Jenkinson & Mandelstam, 1983).

To facilitate the use of phage ϕ 105 and its derivatives as cloning vectors we present a detailed restriction map for 13 enzymes; the ordering of the *EcoRI* fragments is not as presented previously (Perkins *et al.*, 1978; Scher *et al.*, 1978).

METHODS

Bacterial strains. Throughout the work phage ϕ 105 was grown in laboratory stocks of either *B. subtilis* 168 *trpC2* or CU267 (*leuB16 ilvB2 trpC2*). The phages used (ϕ 105 and ϕ 105DI:1t) were originally in strains 1L11 and 1L29 respectively (obtained from the Bacillus Genetic Stock Center, The Ohio State University, Columbus, Ohio, USA).

Isolation of phage and phage DNA. The growth and isolation of phage ϕ 105 and the preparation of phage DNA were according to Jenkinson & Mandelstam (1983).

Restriction endonucleases. These were purchased from Amersham or BRL and were used as described by the manufacturers.

Gel electrophoresis. DNA fragments generated by restriction endonucleases were separated by electrophoresis on agarose gels (Sigma type II; 0.7–1.2%, w/v) in TBE (90 mM-Trizma base, 90 mM-boric acid, 2.5 mM- Na_2EDTA , pH 8.3) or TAE (40 mM-Trizma base, 18 mM-acetic acid, 2 mM- Na_2EDTA , pH 8.1) and visualized by ethidium bromide fluorescence. For preparative gels, low melting point agarose (BRL) was used and DNA fragments were isolated from it as described by the supplier.

The DNA fragments generated by each restriction enzyme were lettered in order of decreasing molecular weight with the largest being fragment B. Phage λ DNA was cleaved with *HindIII* to provide molecular weight markers (Sanger *et al.*, 1982). The sizes of the large restriction fragments were estimated by subtracting the sum of the sizes of the smaller fragments from the size of ϕ 105 DNA.

Table 1. Sizes of fragments generated after digestion of $\phi 105$ DNA with different restriction enzymes

Fragments have been lettered in order of decreasing molecular weight, the largest being fragment B. The lower level of detection of restriction fragments on the gels was about 0.15 kb.

Restriction enzyme	Fragment sizes (kb)															
	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
<i>EcoRI</i>	11.7	9.0	7.6	5.3	3.2	1.05	0.86	0.48								
<i>BglII</i>	29	10														
<i>ClaI</i>	30	9.5														
<i>EcoRV</i>	24	11	4.1													
<i>HindIII</i>	5.6	5.4	4.85	4.1	3.95	2.85	2.8	2.4	1.9	1.2	1.15	0.9	0.85	0.65	0.50	0.17
<i>KpnI</i>	15	14	7.1	3.2												
<i>MluI</i>	12	11	7.1	5.4	3.3											
<i>NcoI</i>	17.5	12	8.3	0.75	0.68											
<i>PstI</i>	6.4	5.1	4.9	4.3	3.6	2.8	2.65	2.3	2.05	1.6*	0.95	0.66	0.33	0.3		
<i>SalI</i>	38	1.5														
<i>SmaI</i>	9.7	8.5	6.3	4.6	4.2	3.0*										
<i>SstI</i>	27	6.9	3.2	2.0												
<i>XhoI</i>	26	9.3	3.7													

* Co-migrating fragments: K1 and K2 for *PstI* and G1 and G2 for *SmaI*.

RESULTS AND DISCUSSION

Phage $\phi 105$ DNA was digested with restriction endonucleases used singly or in combinations of two. The sizes of the fragments generated after digestion with single enzymes are shown in Table 1. Phage $\phi 105$ DNA was found to be resistant to digestion with the restriction endonucleases *BamHI* and *XbaI*; this is in agreement with the results of Jenkinson & Mandelstam (1983).

As shown in Table 1, phage $\phi 105$ DNA was cleaved by *XhoI* to generate three fragments; this is in agreement with the results of Jentsch (1983) but it is in contrast to the observations of Jenkinson & Mandelstam (1983) who did not find any *XhoI* sites. However, the two *XhoI* sites are not in the largest and fourth largest *HindIII* fragments (B and E, respectively) as reported by Jentsch (1983), but in the *HindIII* fragments C and E.

Although Table 1 shows that phage $\phi 105$ DNA possesses one *BglII* site, this enzyme (which has no relaxed specificity or 'star' activity) always produced only partial digestion. The reason for this is not known but it could, for example, be due to secondary structure effects.

A restriction map (Fig. 1) showing the relative positions of the various restriction endonuclease recognition sites on phage $\phi 105$ DNA was obtained using a combination of the following procedures: (i) analysis of DNA fragments obtained after digestion with two or three enzymes; (ii) analysis of *E. coli* and *B. subtilis* plasmids containing phage $\phi 105$ *EcoRI* fragments; (iii) isolation of DNA fragments from low melting point agarose gels and further digestion with one or more restriction endonucleases; and (iv) analysis of the following phages: $\phi 105DI:1t$, $\phi 105 dlys$ and $\phi 105 dspoIIIB$ (Jenkinson & Mandelstam, 1983), $\phi 105DS1$ (Savva & Mandelstam, 1984) and $\phi 105J7$ and $\phi 105J8$ (J. Errington, unpublished).

The 4 kb deletion in phage $\phi 105DI:1t$ (Flock, 1977) lies between the *SmaI* site at 21.05 kb and the *HindIII* site at 25.6 kb.

A number of *EcoRI* fragments of phage $\phi 105$ have been cloned in this laboratory using both *E. coli* and *B. subtilis* plasmids. Restriction enzyme analysis of such plasmids containing the *EcoRI* fragment E or the *EcoRI* fragment I indicates that fragment E contains one *KpnI* site whilst fragment I contains no *KpnI* sites (data not shown). This disagrees with the predictions of the published map for *KpnI* (Jenkinson & Mandelstam, 1983); however, the *KpnI* fragment sizes determined by Jenkinson & Mandelstam (1983) agree with our estimates. When *KpnI* fragment D (6.95 kb) was digested with *EcoRI* only two fragments were obtained (0.65 kb and 6.3 kb). If the order of the *EcoRI* fragments was as shown by Perkins *et al.* (1978) the above reaction should have generated the *EcoRI* fragments G and I; the absence of these fragments confirms the positioning of fragments G and I on the left of fragment E. The relative order of the *EcoRI*

Fig. 1. Restriction map of phage $\phi 105$ DNA. Fragments have been lettered in order of decreasing size, the largest being fragment B. The broken line above the *EcoRI* map indicates the part of the DNA in which the 4 kb deletion of phage $\phi 105$ DI:1t occurs. In the case of *PstI* the relative orientation of fragments K2 and N has not been established. The phage DNA has no recognition sites for the restriction enzymes *BamHI* and *XbaI*. The presence of a small *SstI* fragment (F; about 0.1 kb) was deduced from double digests.

fragments G and I has been determined using partial digestions of $\phi 105$ DNA with *EcoRI*; our data agree with those of Perkins *et al.* (1978). Furthermore, the restriction maps for all the enzymes shown are consistent with the new map order for the *EcoRI* fragments.

The physical map of phage $\phi 105$ of Perkins *et al.* (1978) was based on the partial digestion products produced by *EcoRI*; our ordering of the *EcoRI* fragments is also compatible with these partial digestion products. Mutations in the $\phi 105$ prophage have been placed in a linear order by Armentrout & Rutberg (1970). Three of these mutations *Bsus14*, *Dsus13* and *EtsN9* were found to be rescued by isolated *EcoRI* fragments D, E and G, respectively (Scher *et al.*, 1978); therefore, the putative order of these *EcoRI* fragments (i.e. D, E, G) was inferred from the linear order of the three mutations. Clearly, the new order for the *EcoRI* fragments creates a discrepancy between the physical and genetic maps; the reasons for this are not known.

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