Cloning of the *Bacillus subtilis* *spoIIA* and *spoVA* Loci in Phage ϕ 105DI:1t

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A 6.95 kb *Hind*III-generated DNA fragment from *Bacillus subtilis* 168 was inserted into the DNA of phage ϕ 105DI:1t. The recombinant phage (ϕ 105DS1) contained DNA of 33.8 kb as compared with 35.2 kb for ϕ 105DI:1t and 39.2 kb for the wild-type phage. In the presence of helper phage, ϕ 105DS1 complemented both *spoIIA* and *spoVA* mutations in *B. subtilis*.

INTRODUCTION

Spore formation and germination in *Bacillus subtilis* is controlled by a large number of genetic loci (Young & Mandelstam, 1979; Piggot *et al.*, 1981). The molecular cloning of these loci would help in the identification of their protein products and in the understanding of the regulation of sporulation; also, complementation studies could be performed to establish the number of genes in each of these loci. Recently a number of sporulation genes have been cloned in both phage and plasmid vectors. For example, Hirochika *et al.* (1981) have reported the cloning of *spoOB* in phage ρ 11, Bonamy & Szulmajster (1982) used plasmid pHV33 to clone *spoOB* and *spoIIG* and Jenkinson & Mandelstam (1983) cloned *spoIIIB* in phage ϕ 105.

Earlier reports (Coote, 1972; Piggot, 1973; Akrigg & Mandelstam, 1978; Yudkin & Turley, 1981; Errington & Mandelstam, 1983) have indicated that *spoIIA* is a locus in the main linear sequence of events postulated as governing sporulation (Mandelstam, 1976). Recently, part of *spoIIA* has been cloned in a plasmid (Liu *et al.*, 1982) which was found to complement some, but not all, *spoIIA* mutations. This suggested that the *spoIIA* locus contains at least two genes and, as shown in an accompanying paper (Fort & Piggot, 1984), the number of genes is actually three.

Phage ϕ 105 (Birdsell *et al.*, 1969) has been used to clone a number of *B. subtilis* genes, including *metB*, *lys* and *spoIIIB* (Iijima *et al.*, 1980; Jenkinson & Mandelstam, 1983). In most cases the cloned DNA was about 4 kb and was inserted in the tail region of the ϕ 105 DNA (*Eco*RI fragment E; Perkins *et al.*, 1978). This paper reports the cloning of the *spoIIA* and *spoVA* loci in phage ϕ 105DI:1t (Flock, 1977) and the complementation of *spoIIA* and *spoVA* mutations by the recombinant phage.

METHODS

Bacterial strains. The bacteria and phages used in this investigation are shown in Table 1. Strain SL3030 of *Escherichia coli* carrying plasmid pHM2 (Liu *et al.*, 1982) was provided by P. Piggot.

Buffer solutions. The following buffer solutions were used: (i) TE (10 mM-Trizma/HCl, 1 mM- Na_2EDTA , pH 7.6); (ii) TBE (90 mM-Trizma base, 90 mM-boric acid, 2.5 mM- Na_2EDTA , pH 8.3); (iii) gel loading solution (30%, w/v, Ficoll, 25 mM- Na_2EDTA , 0.25%, w/v, bromophenol blue, 0.25% (w/v) xylene cyanol FF); (iv) SSC (0.15 M- NaCl , 0.015 M-sodium citrate, pH 7.0); (v) Denhardt solution (Denhardt, 1966; 0.2%, w/v, Ficoll, 0.2%, w/v, bovine serum albumin, 0.2%, w/v, polyvinylpyrrolidone in $3 \times$ SSC); (vi) ligase buffer (50 mM-Trizma/HCl, 10 mM- MgCl_2 , 10 mM-DTT, 1 mM-ATP, 1 ng bovine serum albumin ml^{-1} , pH 7.4).

Isolation of phage ϕ 105. Phage ϕ 105 lysates and purified stocks of phage ϕ 105 were prepared as described by Jenkinson & Mandelstam (1983).

Isolation of DNA. Plasmid DNA from *E. coli* was isolated as described by Savva (1983).

Table 1. *Bacterial strains*

<i>B. subtilis</i> strain	Genotype	Origin/Reference
168	<i>trpC2</i>	Laboratory stock
MB75	<i>lys-1 metC3 tal-1</i>	Laboratory stock
CU267	<i>leuB16 ilvB2 trpC2</i>	S. A. Zahler
MY2016	<i>hisH2 lys-1 rpoB2 sul^R</i>	Yudkin & Turley (1980)
566	<i>spoIIA561/spoIIA562</i>	Errington & Mandelstam (1983)
566.5	<i>hisH2 spoIIA561/spoIIA562 rpoB2</i>	566 transformed with DNA from MY2016 to Rif ^R and His ⁻ isolated (I. Lopez-Diaz, this laboratory)
1.1	<i>hisH2 spoIIA1</i>	Errington & Mandelstam (1983)
37.1	<i>hisH2 spoIIA37</i>	Errington & Mandelstam (1983)
69.4	<i>hisH2 spoIIA69</i>	Errington & Mandelstam (1983)
89.7	<i>hisH2 spoVA89 rpoB2 sul^R</i>	Errington & Mandelstam (1984; accompanying paper)
574	<i>hisH2 spoVA574 rpoB2 sul^R</i>	Errington & Mandelstam (1984; accompanying paper)
1L29	<i>trpC2 (φ105DI:1t)</i>	BGSC*
566.6	<i>hisH2 spoIIA561/spoIIA562 rpoB2 (φ105DI:1t)</i>	566.5 lysogenised with φ105DI:1t from 1L29
<i>E. coli</i> strain	Relevant genotype	Origin/Reference
JM83	<i>ara Δ(lac-pro) strA thi φ80lacZ ΔM15</i>	Vieira & Messing (1982)
SL3030	<i>(pHM2) hsdR supE recA56</i>	Liu <i>et al.</i> (1982)

* BGSC, Bacillus Genetic Stock Center, The Ohio State University, Columbus, Ohio, USA.

Chromosomal DNA from *B. subtilis* for use in cloning was prepared according to Marmur (1961); the DNA was finally extracted once with phenol saturated with TE and three times with TE-saturated ether. DNA for use in mapping experiments was prepared as described by Ward & Zahler (1973) from 2.5 ml cultures.

Phage φ105 DNA was extracted essentially according to Jenkinson & Mandelstam (1983) but with the addition of one phenol and three ether extractions at the end.

Transformation and transduction. Transformation of competent *B. subtilis*, transduction of stationary phase *B. subtilis* with phage φ105 and selection of Spo⁺ transformants or transductants were performed as described by Jenkinson (1983) and Jenkinson & Mandelstam (1983). Transformation of *E. coli* and selection of clones carrying recombinant plasmids was done as described by Savva (1983).

Restriction enzymes. Restriction enzymes were obtained from BRL and digestions of DNA were carried out in the buffers recommended by the manufacturer at a concentration of 4 units of enzyme (μg DNA)⁻¹ for 2 h at 37 °C. Reactions were terminated by the addition of Na₂EDTA to 10 mM. Where the DNA was to be used in a ligation reaction, the restriction enzymes were inactivated by heating for 10 min at 70 °C; in the case of *Hind*III (which is not heat-sensitive) the DNA was precipitated for 10 min at room temperature in the presence of 2.5 M-ammonium acetate using 2 volumes of isopropanol (Maniatis *et al.*, 1982).

Electrophoresis of DNA and isolation of restriction fragments from agarose gels. DNA fragments were added to 0.1 volume of gel loading buffer and heated for 5 min at 65 °C before being electrophoresed through 0.8% agarose (Sigma, type II) gels in TBE buffer. The DNA was visualized by ethidium bromide fluorescence.

For preparative gels, low melting point agarose (BRL) was used. The required fragment was cut out of the gel and the agarose was melted at 65 °C for 10 min. The DNA was extracted once with TE-saturated phenol, once with phenol/chloroform (1:1, v/v) and once with chloroform; it was then precipitated in the presence of 1/3 volume 5 M-ammonium acetate and 2.5 volumes 95% ethanol and left at -20 °C for 2 h. The DNA was collected by centrifugation in a Beckman microfuge at 4 °C for 10 min, dried under vacuum and dissolved in an appropriate volume of TE buffer.

Southern blotting and localization of *spoIIA* in digested genomic DNA. Chromosomal DNA was cleaved separately with *Hind*III, *Pst*I or *Sal*I and electrophoresed as described above. The DNA was transferred from the gel to nitrocellulose paper (Schleicher & Schull) using the method of Southern (1975). The filter was heated at 80 °C for 2 h and then incubated at 65 °C for 1 h in 20 ml Denhardt solution containing SDS (0.1%, w/v) and denatured salmon sperm DNA (50 μg ml⁻¹). Hybridizations were performed at 65 °C overnight in the same solution containing 100 ng nick-translated (Rigby *et al.*, 1977) *Bgl*III fragment from the insert of *B. subtilis* DNA in plasmid pHM2 (Liu *et al.*, 1982) at a specific activity of 1 × 10⁷ c.p.m. μg DNA⁻¹. Any probe that did not hybridize was washed off the nitrocellulose paper at 65 °C using two 5 min changes (100 ml) of Denhardt solution containing

0.1% SDS, three 15 min changes of SSC containing 0.1% SDS, two 15 min changes of $0.3 \times$ SSC containing 0.1% SDS and one 5 min change of $3 \times$ SSC. After drying, the filter was autoradiographed at -70°C .

Ligations. The 5.3 kb *Eco*RI fragment of phage ϕ 105 DNA (fragment E; Perkins *et al.*, 1978) was isolated from an agarose gel and 200 ng of it was added to 200 ng *Eco*RI-cleaved plasmid pUC9 DNA (Vieira & Messing, 1982) in 20 μ l ligation buffer; T4 DNA ligase (2 Weiss units; Boehringer) was then added and the reaction mixture was incubated at 15°C for 16 h before being used to transform *E. coli* JM83.

A 10 μ g sample of the pUC9 derivative containing the *Eco*RI fragment E from phage ϕ 105 (pDSMU7; see Results) was cleaved with *Eco*RI and *Hind*III and ligated to 20 μ g *Hind*III-cleaved chromosomal DNA from *B. subtilis* 168; this was used to transform *B. subtilis* 566.6 (lysogenic for phage ϕ 105DI:1t).

RESULTS

Cloning and restriction enzyme analysis of EcoRI fragment E from phage ϕ 105 DNA

In an attempt to increase the probability that two adjacent fragments of ϕ 105 DNA would be ligated to the chromosomal DNA it was decided to reduce the number of phage ϕ 105 *Eco*RI fragments in the ligation reaction by cloning fragment E of ϕ 105 in plasmid pUC9 (Vieira & Messing, 1982) and using this as a source of phage DNA. Plasmid pUC9 codes for ampicillin resistance and a modified α -peptide of *lacZ*; insertion of DNA in the *lacZ* region and transformation of *E. coli* JM83 (*lacZ* Δ M15; Vieira & Messing, 1982) results in the formation of colourless colonies on plates containing X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside). Phage ϕ 105 DNA was cleaved with *Eco*RI and fragment E was isolated from an agarose gel. This was inserted in the *Eco*RI site of plasmid pUC9 and used to transform *E. coli* JM83. A number of colourless clones were obtained and their plasmids were isolated and characterized using restriction enzymes. All the plasmids isolated were indistinguishable; one of them was designated pDSMU7. The sizes of the DNA fragments produced after digestion of pDSMU7 with a number of restriction enzymes are shown in Table 2 and the restriction map of the plasmid is shown in Fig. 1.

Localization of spoIIA in restriction enzyme digested chromosomal DNA

Restriction enzyme analysis of plasmid pHM2 and Tn1000 mutagenesis (Lencastre *et al.*, 1983) had shown that the cloned part of *spoIIA* was present on the 0.7 kb *Eco*RI-*Bgl*II fragment of the *B. subtilis* DNA present in pHM2 (Fig. 2). Accordingly, the *Bgl*II fragment from pHM2 was isolated from an agarose gel, nick-translated and used to probe *Hind*III-cleaved chromosomal DNA as described in Methods. The probe hybridized to three *Hind*III fragments of 0.4, 0.8 and 6.95 kb (Fig. 3) and it was clear, referring to Fig. 2, that *spoIIA* was on the largest of these fragments.

Cloning of spoIIA in phage ϕ 105DI:1t

Plasmid pDSMU7 was cleaved with *Eco*RI and *Hind*III and mixed with *Hind*III-cleaved *B. subtilis* 168 chromosomal DNA. After overnight ligation, the DNA was used to transform *B. subtilis* 566.6 (lysogenic for phage ϕ 105DI:1t) to *Spo*⁺. Twenty-one *Spo*⁺ transformants were obtained and, as discussed by Jenkinson & Mandelstam (1983), these would be a possible source of transducing phage carrying the *spoIIA* locus. Therefore, the 21 *Spo*⁺ transformants were pooled and a lysate was prepared; the resulting lysate had a phage titre of 2×10^9 p.f.u. ml⁻¹. When this lysate was used to transduce strain 566.5 (non-lysogenic for phage ϕ 105DI:1t) to *Spo*⁺, the frequency of *Spo*⁺ transductants was 10^3 per ml of transduced culture. Ten of these *Spo*⁺ transductants were picked and found to be immune to superinfection by phage ϕ 105DI:1t. Therefore, these must have been lysogenised by phage ϕ 105DI:1t carrying the *spoIIA* locus. Lysates were prepared from two of the *Spo*⁺ transductants. These were used to transduce strain 566.5 (non-lysogenic for phage ϕ 105DI:1t) to *Spo*⁺ but no such transductants were obtained. However, when these lysates were supplemented with a lysate of wild-type phage ϕ 105 (1×10^7 p.f.u.) several thousand *Spo*⁺ transductants were obtained, whereas wild-type phage ϕ 105 did not give rise to any when used on its own.

Table 2. *Sizes of the fragments generated after digestion of pDSMU7 with a number of restriction enzymes*

The lower limit of detection of restriction fragments on the gels was about 0.2 kb.

Restriction enzyme(s)	Fragment sizes (kb)						
<i>Kpn</i> I	8.0						
<i>Eco</i> RI	5.25	2.7					
<i>Eco</i> RI, <i>Kpn</i> I	4.65	2.7	0.61				
<i>Sma</i> I	4.46	2.93	0.67				
<i>Eco</i> RI, <i>Sma</i> I	2.93	2.7	1.76	0.67			
<i>Kpn</i> I, <i>Sma</i> I	4.45	2.93	0.61				
<i>Hind</i> III	3.7	2.93	0.94	0.51			
<i>Eco</i> RI, <i>Hind</i> III	2.93	2.7	1.0	0.94	0.51		
<i>Kpn</i> I, <i>Hind</i> III	3.7	2.93	0.83	0.51			
<i>Sma</i> I, <i>Hind</i> III	3.7	2.18	0.8	0.76	0.51		
<i>Eco</i> RI, <i>Hind</i> III, <i>Sma</i> I	2.7	2.18	1.0	0.8	0.76	0.51

Fig. 1. Restriction map of the plasmid pDSMU7. The *Eco*RI site of pUC9 is used as the origin for map distances (kb) which are shown inside the innermost circle. Restriction sites for *Eco*RI, *Hind*III, *Sma*I and *Kpn*I are shown in the concentric circles; the stippled area represents the parent plasmid.

Complementation of spoIIA and spoVA mutations

The lysate from one of the *Spo*⁺ transductants (this strain was designated SG32) was supplemented with wild-type phage ϕ 105 (1×10^7 p.f.u. ml⁻¹) and 0.1 ml portions were used to transduce strains 69.4, 37.1 and 1.1 to *Spo*⁺. All three strains were found to give rise to *Spo*⁺ transductants at a frequency of 1×10^4 per ml of transduced culture.

As shown by Piggot *et al.* (1984; accompanying paper) plasmid pPP33 contains a *Bgl*III fragment of *B. subtilis* DNA which transforms those *spoIIA* mutations that are not complemented by pHM2; it also transforms some, but not all, *spoVA* mutations (J. Errington, personal communication). It was, therefore, expected that the recombinant phage in the above lysate would transduce *spoVA* mutations to *Spo*⁺. Consequently, the lysate was used to transduce strains 574 and 89.7 to *Spo*⁺ and both were found to be transduced to *Spo*⁺ at the same frequency as the *spoIIA* strains.

It was necessary to check that the *Spo*⁺ transductants had arisen through complementation of the *spoIIA* or *spoVA* mutations and not through recombination in the *spoIIA* or *spoVA* regions of the chromosome. To show that the *spo* allele was still present in the *Spo*⁺ transductants, DNA

Fig. 2. Restriction map of the plasmid pHM2 according to Liu *et al.* (1982). Map distances (kb) are shown inside the innermost circle. Restriction sites for *Hind*III, *Eco*RI, *Sal*I, *Pst*I and *Bgl*II are shown in the concentric circles. The region of *B. subtilis* DNA is outlined in bold and the *spoIIA* part of the insert (Lencastre *et al.*, 1983) is stippled.

was isolated from five *Spo*⁺ transductants from strains 69.4 or 89.7 and each DNA preparation was used at a saturating concentration to transform strain MB75 to *Lys*⁺. In such an experiment, about 30% of the *Lys*⁺ transformants would be expected to acquire the *spoIIA69* mutation (Yudkin & Turley, 1981) and about 40% would be expected to acquire the *spoVA89* mutation (Errington & Mandelstam, 1984; accompanying paper); *Spo*⁻ colonies were identified by their failure to produce the characteristic brown pigment associated with sporulation. Because the results with the five different DNA preparations did not differ significantly, only the combined values are quoted. Among the *Lys*⁺ transformants obtained using DNA from the *Spo*⁺ transductants of strain 69.4, 26% (1106/4219) were *Spo*⁻, while among those obtained using DNA from the *Spo*⁺ transductants of strain 89.7, 36% (1270/3519) were *Spo*⁻. It was concluded that the *Spo*⁺ transductants still carried their respective *spo* alleles.

The phage isolated from *B. subtilis* SG32 has been designated ϕ 105DS1.

Restriction enzyme map of ϕ 105DS1 DNA

The restriction enzyme *Eco*RI has been shown to cleave phage ϕ 105 DNA into nine fragments which were lettered B to J in order of decreasing size and arranged into a linear map by Perkins *et al.* (1978); this nomenclature has been maintained in this paper. The fragments obtained after digestion of phage ϕ 105 DNA with *Eco*RI are shown in Fig. 4 (lane 2). Phage ϕ 105DI:1t has a 4 kb deletion in fragment B and consequently fragments B' and D co-migrate during electrophoresis as shown by the staining intensity (Fig. 4, lane 3). The sums of the molecular weights of the *Eco*RI fragments of phages ϕ 105 and ϕ 105DI:1t were 39.2 and 35.2 kb, respectively. Fragment J (about 0.35 kb) was not among the digestion products and the absence of this band was also noted by Iijima *et al.* (1980) and Jenkinson & Mandelstam (1983).

Digestion of DNA from phage ϕ 105DS1 with *Eco*RI resulted in the disappearance of fragments B' and E and the appearance of a fragment co-migrating with fragment C (shown by the staining intensity of the bands) and a 2.1 kb fragment (Fig. 4, lane 4). The sum of the molecular weights of the fragments obtained after digestion of phage ϕ 105DS1 DNA with *Eco*RI was 33.8 kb.

Fig. 3. Hybridization of ^{32}P -labelled *Bg*/II fragment from pHM2 to *Hind*III-cleaved *B. subtilis* 168 DNA. The Figure is a composite of the photographs of the autoradiograph (left) and of the molecular weight markers (*Hind*III-cleaved phage DNA; right) which were electrophoresed on the same gel and visualized by ethidium bromide fluorescence before the DNA was blotted from the gel onto the nitrocellulose filter. The sizes of the molecular weight markers are given in kb and the origin is marked.

Fig. 4. Analysis of DNA from phages $\phi 105\text{DI:1t}$ and $\phi 105\text{DS1}$ with *Eco*RI and *Hind*III. The digested DNA samples were electrophoresed on a 0.8% agarose gel, stained with ethidium bromide and photographed. Lanes 1 and 7, phage λ *Hind*III fragments with sizes given in kb; lane 2, phage $\phi 105$ *Eco*RI fragments; lane 3, $\phi 105\text{DI:1t}$ digested with *Eco*RI; lane 4, $\phi 105\text{DS1}$ with *Eco*RI; lane 5, $\phi 105\text{DS1}$ with *Hind*III; lane 6, $\phi 105\text{DI:1t}$ with *Hind*III.

Comparison of the fragments generated by the digestion of $\phi 105\text{DS1}$ and $\phi 105\text{DI:1t}$ DNAs with *Hind*III shows that the former contained a 6.95 kb fragment instead of the 4.72 and 0.96 kb fragments (Fig. 4, lanes 5 and 6); the loss of these two fragments was the result of a deletion in the right-hand side of the *Eco*RI fragment E and the left-half side of the *Eco*RI fragment B'. Southern hybridizations of these *Hind*III fragments with nick-translated *Bg*/II fragment of

ϕ 105DI:1t

Fig. 5. Restriction maps of DNA from phages ϕ 105DI:1t and ϕ 105DS1. The restriction sites shown on the enlarged version of the cloned fragment of *B. subtilis* DNA carrying the *spoIIA* and *spoVA* loci (represented by the solid line in ϕ 105DS1) agree with those published for pHM2 (Liu *et al.*, 1982) and pPP33 (Piggot *et al.*, 1984; accompanying paper); they also agree with the DNA sequence data for *spoIIA* (Fort & Piggot, 1984; accompanying paper). The *EcoRI* fragments of phage ϕ 105DI:1t are lettered B' to I; *HindIII* sites (H) are shown only for fragment E. Bc, *BclI*; Bg, *BglII*; C, *ClaI*; E, *EcoRI*; K, *KpnI*; P, *PstI*; Pv, *PvuII*; Sm, *SmaI*; V, *EcoRV*.

Table 3. Sizes of the fragments generated after digestion of the 6.95 kb *HindIII* fragment with various restriction enzymes

The lower limit of detection of restriction fragments on the gels used was about 0.2 kb.

Restriction enzyme(s)	Fragment sizes (kb)								
<i>ClaI</i>	5.1	1.8							
<i>EcoRI</i>	5.0	0.98							
<i>SmaI</i>	5.6	1.3							
<i>EcoRI, ClaI</i>	5.15	0.98	0.8						
<i>EcoRI, SmaI</i>	4.65	1.3	0.98						
<i>BclI</i>	4.2	2.4	0.4						
<i>BglII</i>	3.4	1.9	1.68						
<i>PstI</i>	2.7	2.3	1.95						
<i>EcoRI, BclI</i>	4.2	1.4	0.98	0.4					
<i>EcoRI, BglII</i>	3.4	1.9	0.98	0.7					
<i>EcoRI, PstI</i>	2.3	1.95	1.75	0.98					
<i>SmaI, BglII</i>	3.4	1.68	1.25	0.65					
<i>EcoRV</i>	2.85	1.95	1.25	0.9					
<i>EcoRI, EcoRV</i>	1.95	1.80	1.25	0.98	0.9				
<i>PstI, EcoRV</i>	2.75	1.95	1.25	0.7					
<i>PvuII</i>	1.78	1.49	1.1	0.9	0.54	0.44	0.41	0.3	
<i>EcoRI, PvuII</i>	1.78	1.1	0.98	0.9	0.54	0.5	0.44	0.41	0.3
<i>PstI, PvuII</i>	1.49	1.15	1.1	0.75	0.65	0.54	0.44	0.41	0.3

plasmid pHM2 (Fig. 2) indicated that the *spoIIA* locus was present on the 6.95 kb fragment (results not shown).

Analysis of phage ϕ 105DS1 DNA with a number of restriction enzymes (results not shown) has enabled us to construct the physical map of the new phage (Fig. 5). The positions of the

EcoRI fragments G and I are not as reported by Perkins *et al.* (1978) but according to the new restriction map of phage $\phi 105$ (Bugaichuk *et al.*, 1984).

The 6.95 kb *HindIII* fragment from phage $\phi 105DS1$ was isolated from a low melting point agarose gel and subjected to single or double digestion with a number of restriction enzymes. The sizes of the resulting fragments are shown in Table 3. Figure 5 shows the positions of the various restriction enzyme recognition sites on the cloned fragment of *B. subtilis* DNA.

DISCUSSION

Recently a number of investigators have reported the cloning of *B. subtilis* DNA in phage $\phi 105$ (see Introduction). In most cases, the cloned DNA was about 4 kb and it was inserted into the phage chromosome by substitution of part or all of the *EcoRI* fragment E giving rise to tail-less phages. To reduce the number of phage DNA fragments present in the ligation (and therefore increase the efficiency of cloning) it was decided to clone fragment E of $\phi 105$ in plasmid pUC9 (Vieira & Messing, 1982) and use this as a source of phage fragments. One such plasmid (pDSMU7) was isolated and characterized using restriction enzymes.

Using plasmid pHM2 (Liu *et al.*, 1982) and Southern hybridizations the *spoIIA* locus was shown to reside in a 6.95 kb *HindIII* fragment of *B. subtilis* DNA. This fragment was larger than any other fragment previously cloned in phage $\phi 105$. One possible reason for the previous failure to clone fragments larger than 4 kb could be the packaging restrictions imposed by the phage. If this is correct it should be possible to clone larger fragments by the use of a deletion mutant of phage $\phi 105$. It was therefore decided to use phage $\phi 105DI:1t$ (Flock 1977) which has a 4 kb deletion in a non-essential region of the phage chromosome (*EcoRI* fragment B).

As expected, the recombinant phage $\phi 105DS1$ had lost its plaque-forming ability but none of its other functions were affected; similar observations were made by Iijima *et al.* (1980) and Jenkinson & Mandelstam (1983). Like these authors, we found on electron microscopic examination of the phage that no tails were present (results not shown). When *B. subtilis* lysogenic for phage $\phi 105DS1$ was induced with mitomycin C only one type of $\phi 105$ phage could be isolated; this is in agreement with the observations of Jenkinson & Mandelstam (1983) but in contrast to those of Iijima *et al.* (1980). Phage isolated from lysogenic cells after several subcultures was found to be identical with the original isolate (results not shown) indicating that phage $\phi 105DS1$ was stably maintained as a prophage.

Comparison of the fragments generated after the digestion of DNA from phages $\phi 105DI:1t$ and $\phi 105DS1$ with *EcoRI* indicates that large portions of the *EcoRI* fragments B' and E have been deleted. Although deletion of DNA from fragment E might have resulted from the construction of the phage, the reasons for the loss of the right-hand extremity of this fragment and most of fragment B' are not known. Presumably, this deletion arose during the induction and packaging of the recombinant phage.

Integration of phage $\phi 105DS1$ into the *B. subtilis* chromosome allows complementation studies to be carried out. Liu *et al.* (1982) showed that *spoIIA* mutations at one end of the locus could be complemented by plasmid pHM2; transduction with phage $\phi 105DS1$ indicated that *spoIIA* mutations at both ends of the locus could be complemented. Phage $\phi 105DS1$ was also found to complement *spoVA* mutations which could not be complemented in protoplast fusion experiments (Dancer & Mandelstam, 1981). The fact that complementation was obtained by using the phage shows that mutations in *spoVA* are recessive and that the earlier indeterminate result was due to some inherent limitation of the protoplast fusion technique. It is apparent that phage $\phi 105$ and its deletion mutants are useful not only for cloning in *B. subtilis* but also for complementation studies.

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