The Isolation of λ Phage Carrying DNA from the Histidine and Isoleucine-Valine Regions of the *Bacillus subtilis* Chromosome

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From a λ gtWES library of the chromosome of *Bacillus subtilis*, phages carrying DNA from the *hisA* and *ilv-leu* regions were isolated. They were identified by their ability to form complementing plaques on *hisB*, *ilvC* or *leuB* mutants of *Escherichia coli* K12 under selective conditions and in the presence of a helper phage. The *his* phages complemented *E. coli hisA*, *B* or *D* mutations and could transform seven mutations in the *hisA* region of the *B. subtilis* chromosome; each carried a single *EcoRI* insert of about 8.2 kb. Phages complementing *E. coli ilvC* or *leuB* mutations and carrying the equivalent *B. subtilis* genes *ilvC* and *leuC* transformed a range of mutations in the *B. subtilis ilv-leu* region. The distribution of genetic markers carried by the phages suggests that the entire *ilv-leu* cluster from *azlA* through *leuD* is covered in the collection of phages obtained and is carried in three *EcoRI* restriction fragments of approximately 6.7, 4.7 and 2.85 kb.

INTRODUCTION

The expression of *Bacillus subtilis* genes cloned in *Escherichia coli* plasmid or phage vectors can be exploited in selection procedures based on complementation of *E. coli* defects. *leuB* and *pyrE* mutations of *E. coli* were complemented in cells lysogenic for a λ vector carrying *B. subtilis* DNA (Chi *et al.*, 1978), and the β -lactamase from *Bacillus licheniformis* (Brammar *et al.*, 1980) was also expressed in *E. coli*. Multicopy plasmids carrying the *B. subtilis leuC* gene can also complement an *E. coli leuB* mutant (Mahler & Halvorson, 1977; Nagahari & Sakaguchi, 1978). Some instability of cloned *B. subtilis* fragments maintained in *B. subtilis* has been encountered, with both phage and plasmid vectors (Kreft & Hughes, 1982); one advantage of cloning in *E. coli* is that the cloned DNA may be more stable. The fumarase gene of *B. subtilis* was detected in a λ library by the formation of microcolonies of weakly complementing lysogens in phage plaques (Moir, 1983). This paper describes the extension of this plaque complementation approach to the isolation of other *B. subtilis* genes. The *his* and *ilv-leu* gene clusters were chosen since not only is their molecular genetics and regulation important but they are also closely linked to spore germination (*ger*) mutations in the *F* and *E* and *H* loci respectively (Piggot *et al.*, 1981). Cloning of *his* and *ilv-leu* should facilitate the detection of nearby sequences concerned with spore germination.

Very little is known about the genetic control of histidine biosynthesis in *B. subtilis*. Mutations affecting histidine biosynthesis in *B. subtilis* map either at *hisA* (305°) or *hisH* (205°) (Henner & Hoch, 1980). The *hisH* locus forms part of a cluster of genes concerned with aromatic amino acid biosynthesis and encodes histidinol phosphate aminotransferase (Nester & Montoya, 1976; Borenstein & Ephrati-Elizur, 1969). All other *B. subtilis his* mutations described map in the *hisA* region (Henner & Hoch, 1980), so that most of the *his* genes may be located in this region.

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At least part of this *hisA* gene cluster has been cloned in phage $\rho 11$ (Kawamura *et al.*, 1979). The transduced fragment, however, may have already suffered an internal 1.6 MDal deletion, either during cloning or in the subsequent isolation of plaque-forming phage (Kawamura *et al.*, 1981). The *his* insert in $\rho 11$ was not stable, and was lost in approximately 5% of progeny phage (Mizukami *et al.*, 1980; Kawamura *et al.*, 1981).

The *ilv-leu* gene cluster is one of the best-characterized potential operons of *B. subtilis*. Genetical and enzymological studies of mutants and an examination of the regulation of expression of the genes (Ward & Zahler, 1973*a, b*) have been performed. The isolation of SP β defective *ilv*- and *leu*-transducing phages facilitated complementation analysis but they were unsuitable for isolation of the DNA of the region (Mackey & Zahler, 1982). An *EcoRI* fragment of 4.2 MDal which includes *leuC* has been cloned on a plasmid vector (Nagahari & Sakaguchi, 1978) and λ (Chi *et al.*, 1978). It carries *leuA*, *B* and *C* alleles, but not *ilvB* or *ilvC* (Nagahari & Sakaguchi, 1978) and since its *leuA* and *C* functions provide only weak complementation even in *B. subtilis*, it is likely that the natural promoter is absent (Tanaka & Sakaguchi, 1978).

The plaque-complementation approach has permitted isolation and preliminary characterization of more extensive fragments of DNA from the *hisA* and *ilv-leu* regions.

METHODS

Bacteria. The strains of *E. coli* K12 and *B. subtilis* 168 used are listed in Table 1.

Phages. A library constructed by Drs E. Ferrari and J. A. Hoch by ligation into the purified arms of the vector λ gtWES. λ B (Leder *et al.*, 1977) of a 10–13 kb fraction of the products of partial *EcoRI* digestion of *B. subtilis* 168 chromosomal DNA was used. This was propagated once on ED8654 to permit methylation by the *E. coli* K modification system.

λ imm⁴³⁴Ram54am60 (Moir & Brammar, 1976) was used as helper phage. Transducing phages carrying *B. subtilis* DNA were named λ BS*his*, λ BS*ilv* or λ BS*leu* according to the selection used to isolate them.

Media. (a) *E. coli*. Strains were cultured in L-broth and on L-agar (Miller, 1972). Minimal medium for the complementation experiments was M9 salts (Miller, 1972) supplemented with MgSO₄ (10 mM), glucose (0.4%), maltose (0.4%) and an amino acid supplement based on the AA medium of Tanaka & Sakaguchi (1978) which comprised 50 μ g ml⁻¹ each of alanine, arginine, aspartic acid, cysteine, sodium glutamate, glycine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and tyrosine. In addition, guanine, histidine and leucine were added for *ilv* selection, isoleucine, leucine and valine for *his* selection and histidine, isoleucine, valine, thymine (all at 50 μ g ml⁻¹) and thiamin (5 μ g ml⁻¹) for *leu* selection. For complementing phage plaques, this medium, further supplemented with 1–2 ml L-broth per litre, was solidified with 1% (w/v) Difco Bacto Agar to form bottom agar; top agar was the same medium without L-broth and with 0.65% Difco Bacto Agar. Phage was propagated and titrated on BBL agar (Parkinson, 1968).

(b) *B. subtilis*. Nutrient media were Difco Penassay Broth and Potato Glucose Yeast Extract Agar (Dring & Gould, 1971). Minimal agar (Anagnostopoulos & Spizizen, 1961) was supplemented with glucose (0.5%) and amino acids (50 μ g ml⁻¹) where required. Culture of *cit* mutants and selection of Cit⁺ was according to Rutberg & Hoch (1970).

λ phage techniques. Preparation of plating cells, phage propagation, purification and reisolation from single plaques, phage preparation and assay have all been described (Murray *et al.*, 1973). Phages were tested for the presence of suppressible mutations by spotting suspensions (at about 10⁵ p.f.u. ml⁻¹) on lawns of Ymel, W1485E and W3350, and tested for immunity on lawns of ED8654(λ) and ED8654(λ imm⁴³⁴). Plating cells for complementation experiments were prepared in L broth + maltose (0.4%). Complementation of *E. coli his*, *leu* or *ilv* auxotrophs was determined by spotting 10 μ l of lysates onto lawns of the appropriate strain on supplemented M9 medium; more dense growth of lysogens within the spot after 2–4 d at 30 °C indicated complementation.

Complementation of *E. coli* mutants by mixed infection. This was a modification of the method of Moir (1983). Auxotrophic recipients were strains 4930, JA300 and CGSC2851. The multiplicity of infection by library and helper phages varied as described in Results. Incubation was 4–6 d at 30 °C, after which complementing plaques or clusters of microcolonies could be observed. Transducing phages were purified by one or two further cycles of plaque formation on selective and one on non-selective medium. Phage lysates sufficiently concentrated for use as donor in transformation could be prepared from suspensions of single complementing plaques by plating 0.1–0.3 ml with strain ED8654 on L-agar.

Transformation. Transformation of *B. subtilis* was as described previously (Anagnostopoulos & Spizizen, 1961; Warburg & Moir, 1981). λ hybrid phages were used directly in transformation by substituting up to 0.1 ml lysate for 0.1 ml DNA (Ferrari *et al.*, 1981). The spore germination (Ger) phenotype was scored by the tetrazolium overlay method (Trowsdale & Smith, 1975; Lafferty & Moir, 1977).

Table 1. *Bacterial strains*

Strain	Genotype	Source	Reference
<i>E. coli</i> K12			
4930	<i>hisB463 λ⁻ F⁻</i>	E. coli Genetic Stock Center	Struhl <i>et al.</i> (1976)
5388	<i>hisA323 λ⁻ HfrG6</i>		
6401	<i>hisD4758 λ⁻ F⁻</i>		Goldschmidt <i>et al.</i> (1970)
CGSC2851	<i>proA2 aroA357 his4 ilvC7 argE3 thi-1 lacY1 galK2 xyl-7 mtl-2 trf-3 tsx-358 supE44</i>		
CV437	<i>leuA371 Δ(pro-lac) thi λ⁻ F⁻</i>		
CV522	<i>leuC222 F⁺</i>	J. Guest S. A. Zahler	Murray <i>et al.</i> (1977)
CV526	<i>leuD101 F⁺</i>		
ED8654	<i>supE supF hsdR⁻ M⁺ S⁺ met trpR</i>		
JA300	<i>trp thi thr thy leuB6 hsdR hsdM</i>		
W1485E	<i>supE</i>		
W3350	<i>sup^o</i>	W. J. Brammar	
Ymel	<i>supF</i>		
<i>B. subtilis</i> 168			
1477	<i>hisA1 cysB3 trpC3</i>	C. Anagnostopoulos	
1604	<i>trpC2</i>	This laboratory	Moir (1981)
4734	<i>leuA8 gerE36</i>		
4735	<i>ilvBΔ1 gerE36</i>		
4736	<i>ilvB2 gerE36</i>	L. Rutberg	Moir (1981)
4822	<i>citF83 leuC2</i>		
4999	<i>hisA1 gerF21 trpC2</i>	This laboratory	
5176	<i>hisA1 gerF99 trpC2</i>		
5185	<i>leuB16 gerH1</i>		
CU116	<i>hisA3 trpC2</i>	S. A. Zahler	
CU223	<i>hisA10 trpC2</i>		
CU267	<i>ilvB2 leuB16 trpC2</i>		
CU315	<i>leuD117 trpC2</i>		
CU431	<i>hisA11 trpC2</i>		
CU697	<i>hisA697 trpC2</i>		
CU511	<i>hisA511 trpC2</i>		
CU677	<i>hisA677 trpC2</i>		

DNA preparation. For initial restriction mapping of some phages the method for isolation of *λ* DNA was that of Sammons & Anagnostopoulos (1982). Large-scale preparations were made by the liquid lysate method of Loenen & Brammar (1980). *λ*ilv and *λ*leu phages were purified on CsCl step gradients, and DNA liberated by formamide (Davis *et al.*, 1980). *λ*his and *λ*leu phages were treated with RNAase and DNAase and precipitated with polyethylene glycol 6000. After 15 h at 4 °C, the precipitate was centrifuged at 10000 *g* (20 min) and the pellet resuspended in 5 ml 50 mM-Tris/HCl + 20 mM-EDTA and 0.1% SDS at pH 7.5. The polyethylene glycol was extracted with an equal volume of chloroform, the supernatant centrifuged at 65000 *g* (1 h) and the pellet resuspended in 1.5 ml of the same Tris buffer. DNA was purified by phenol extraction (Murray *et al.*, 1977) and precipitated twice in two volumes of chilled ethanol (−20 °C) before resuspension in 200–500 μ l buffer (100 mM-Tris/HCl pH 7.2 + 5 mM-MgCl₂ and 50 mM-NaCl).

B. subtilis chromosomal DNA for transformation was prepared by the method of Marmur (1961).

Restriction enzymes. *Eco*RI and *Bam*HI (Bethesda Research Laboratories) were used according to the manufacturer's instructions.

Gel electrophoresis. Electrophoresis of DNA fragments in agarose gels was as described by Moir (1983).

RESULTS

Isolation of transducing phages

Complementation of a mutation in *E. coli* by *λ* hybrid phage carrying *Bacillus* DNA can be detected either by the production of lysogenic transductants (Chi *et al.*, 1978; Brammar *et al.*, 1980) or by the increased growth of lysogens within plaques (Moir, 1983). The latter method has the advantage that even if individual lysogens grow poorly they may be detected as clusters of microcolonies in the centre of plaques. The helper phage *λ*imm⁴³⁴Ram54am60 complements mutations in essential *λ* genes *W*, *E* and *S* and provides homology for integration of the *λ*gtWES

prophage (Moir, 1983). Transducing plaques on a lawn of auxotrophic cells (Franklin, 1971) can appear as lysogen-filled; surrounding cells may be cross-fed, so that the edges of the plaque can be discerned.

Mixed infection of the histidine (*hisB*) auxotroph 4930 gave seven *his*-complementing plaques (λ BS*hisA*I–VII inclusive) when 2×10^7 cells were infected with library and helper phages at multiplicities of infection of 7 and 14 respectively. A second experiment yielded two *his*-complementing plaques when 2×10^7 cells were infected with library and helper phages at multiplicities of infection of 0.5 and 1.4 respectively. Phages derived from one of these plaques (λ BS*hisA*VIII) were studied further.

λ BS*leu* phages were isolated from a complementation experiment in which JA300 cells were infected at a multiplicity of 1 with library and 3 with helper phage. A 10 μ l sample of the mixture containing 3×10^6 infected cells was plated on selective medium with 0.1–0.2 ml of plating cells (10^9 ml⁻¹). From this inoculum eight clusters of microcolonies were obtained, five of which were in clearly visible plaques. Phages isolated from all eight transformed strain CU267 to *leuB*⁺. In this selection a second type of more weakly complementing activity was detected at a 10-fold greater frequency. These plaques contained phages unable to transform *B. subtilis leu* mutations and were not studied further. In an analogous experiment, complementation of strain CGSC2851 gave a total of eight *ilv*-complementing, lysogen-containing plaques from four plates, each seeded with 2.5×10^6 cells infected at a multiplicity of 2 of each type of phage per cell. The frequency of transduction was thus 2.7×10^{-6} – 3.5×10^{-7} per infected cell across this range of experimental conditions.

Phage isolates were purified by repeating the initial selective procedure and then plating non-selectively. Transducing and non-transducing phages were obtained, as defined by the capacity to transform equivalent *B. subtilis* mutations. Immune specificity alone was insufficient to distinguish them, as recombinants which had exchanged immunity regions were common amongst the progeny phages in the complementing plaques.

Characterization of λ BS*hisA* phages

All of the eight λ BS*hisA* phages tested complemented *E. coli hisA*323 and *D4758* mutations in addition to *hisB*463. They also all transformed seven *B. subtilis his* mutations which map in the *hisA* region (S. A. Zahler, personal communication). Representative data for two isolates are shown in Table 2. On the basis of their phage immunity and facility to plaque on *supE* and *F*, *supE* and suppressor-free strains, the phages fell into three groups: (i) λ BS*hisA*I, II, III, IV, VI and VII – *imm*⁺ and retaining at least the *S* mutation; (ii) λ BS*hisA*V – *imm*⁴³⁴ and lacking *W*, *E* and *S* mutations; (iii) λ BS*hisA*VIII – *imm*⁺ and retaining either or both the *W* and *E* mutations. Recombination with helper phage during the mixed infection could have resulted in such an exchange of markers.

Six of these λ BS*his* isolates (the exceptions being IV and VII) were screened by *Eco*RI digestion of small-scale DNA preparations. All contained a single *Eco*RI insert of 8.2 kb, and isolate V had a restriction pattern consistent with the introduction of an additional *Eco*RI site in the *imm*⁴³⁴ region. A simple restriction map was constructed for λ BS*hisA*I, II and III. The results for each were identical and are summarized in Fig. 1. The right arm of the phage retains the mutations to *Eco*RI restriction-resistance and the *nin5* deletion; the insert contains a single *Bam*HI target towards its right hand end (showing that its orientation is the same in all three cases), and four *Hind*III sites.

Characterization of λ BS*leu* and λ BS*ilv* phages

A large number of isolates were obtained from several *leu* and *ilv* complementation experiments. Initial confirmation of transforming activity was obtained by transformation of *B. subtilis* CU267 to *Leu*⁺ or *Ilv*⁺ respectively with impure lysates prepared from complementing plaques. Selection of the alternative marker revealed that 9 out of 12 *leu*-complementing phages tested also carried *ilvB*2⁺ and 10 of 27 *ilv*-complementing phages tested carried *leuB*16⁺. All isolates complementing *E. coli ilvC*, and thus carrying the entire *ilvC* gene of *B. subtilis*, also

Table 2. Transformation of *B. subtilis* auxotrophs by λ BS*his*, λ BS*ilv* and λ BS*leu* phages

The *B. subtilis* strains were transformed to His⁺, Ilv⁺ or Leu⁺, using preparations of whole phage as donor. The competence of the recipients was indicated by the efficiency of transformation using control strain 1604 (*trpC2*) chromosomal DNA.

Donor	No. of transformants ml ⁻¹						
	Recipient strain:						
	1477 <i>hisA1</i>	CU116 <i>hisA3</i>	CU223 <i>hisA10</i>	CU511 <i>hisA511</i>	CU431 <i>hisA11</i>	CU677 <i>hisA677</i>	CU697 <i>hisA697</i>
None	50	20	<10	<10	<10	60	<10
λ^+ (10 ⁹ p.f.u.)	<10	70	<10	<10	<10	<10	<10
λ BS <i>hisA1</i> (2 × 10 ⁸ p.f.u.)	3 × 10 ⁴	7 × 10 ³	3 × 10 ⁴	3 × 10 ²	7 × 10 ³	4 × 10 ²	3 × 10 ³
λ BS <i>hisA11</i> (9 × 10 ⁸ p.f.u.)	>10 ⁵	5 × 10 ⁴	>10 ⁵	10 ⁴	10 ⁵	2 × 10 ⁴	3 × 10 ⁴
1604 (0.09 µg DNA)	6 × 10 ³	10 ⁴	6 × 10 ⁴	7 × 10 ²	5 × 10 ⁴	8 × 10 ³	3 × 10 ³

Donor	Recipient strain:			
	CU267		4735	4734
	<i>ilvB2</i>	<i>leuB16</i>	<i>ilvBΔ1</i>	<i>leuA8</i>
None	<10 ²	<10 ²	<10 ²	<10 ²
λ BS <i>ilv1</i> (5 × 10 ⁷ p.f.u.)	5.1 × 10 ⁴	<10 ²	<10 ²	<10 ²
λ BS <i>ilv2</i> (5 × 10 ⁷ p.f.u.)	1.3 × 10 ⁵	<10 ²	7.2 × 10 ³	10 ²
λ BS <i>leu3</i> (4.6 × 10 ⁷ p.f.u.)	<10 ²	6 × 10 ⁴	<10 ²	5.3 × 10 ⁴
λ BS <i>leu104</i> (1 × 10 ⁸ p.f.u.)	1.7 × 10 ⁵	1.2 × 10 ⁵	10 ²	6.5 × 10 ⁴
1604 (0.1 µg DNA)	2.5 × 10 ⁴	3.3 × 10 ⁴	2.3 × 10 ³	1.3 × 10 ⁴

Table 3. Identification of the *B. subtilis* genes carried by λ BS*leu* and λ BS*ilv* phages

λ BS*leu* and λ BS*ilv* phage lysates were used to transform *B. subtilis* or complement *E. coli* *leu* and *ilv* auxotrophs. The properties of phages representative of the four types obtained are presented: +, repairs *B. subtilis* lesion by recombination; −, fails to repair *B. subtilis* lesion by recombination; (+), complements equivalent *E. coli* gene; (−), fails to complement equivalent *E. coli* gene.

Lysate	<i>B. subtilis</i> map locations:						
	<i>ilv</i>		<i>leu</i>				
	<i>azlA</i>	<i>B2</i>	<i>C</i>	<i>A8</i>	<i>C2</i>	<i>B16</i>	<i>D117</i>
<i>ilvBΔ1</i> :
λ BS <i>leu3</i>	−	−	(−)	(+)	(+)	(+)	+
λ BS <i>leu104</i>	−	+	(+)	(+)	(+)	(+)	+
λ BS <i>ilv1</i>	−	+	(+)	−	(−)	−	−
λ BS <i>ilv2</i>	+	+	(+)	−	(−)	−	−

transformed *ilvB2*. The results of the extensive transformation experiments using purified lysates are shown in Tables 2 and 3.

Crosses with *B. subtilis* strain 4735 determined whether DNA covering the *ilvBΔ1* deletion was present. Seven of the λ BS*ilv* but none of the λ BS*leu* phages tested carried *ilvBΔ1*⁺. Phages complementing the *E. coli* *leuB* gene must carry a functional copy of the equivalent *B. subtilis* gene *leuC*. Such λ BS*leu* isolates could repair by recombination *leuA8*, *leuC2*, *leuB16* and *leuD117* mutations (Table 3). As examples the ability of λ BS*leu3* and λ BS*leu104* to complement the *E. coli* *leuA*, *leuC* and *leuD* defects in strains CV437, CV522 and CV526 were tested. The *E. coli* *leuA* and *B. subtilis* *leuA* genes are equivalent in function (Ward & Zahler, 1973a). The *leuC* and *leuD* genes of *E. coli* encode the two subunits of isopropylmalate isomerase, and it is probable that the *B. subtilis* genes *leuB* and *leuD* are both required to encode the equivalent *B. subtilis* enzyme (Mackey & Zahler, 1982). Complementation of the *E. coli* *leuA* and *leuC* defects was obtained but that of *leuD* was not detected in the spot test method used.

Fig. 1. Simple restriction maps of λ^+ , λ gtWES. λ B and λ BShis, λ BSleu and λ BSilv derivatives. The numbers indicate fragment sizes (kb) which were either estimated by comparison with standards or obtained from a map of λ DNA (Szybalski & Szybalski, 1979). Dashed arrows, *Bam*HI sites; solid arrows, *Eco*RI sites; hatched areas, deleted regions; zig-zag lines, inserted *B. subtilis* DNA.

Restriction maps (Fig. 1) of λ BSilv and *leu* phages representative of each of four classes based on patterns of recombination and complementation (Table 3) were constructed. The restriction pattern was complicated by the presence of *imm*⁴³⁴, *Eco*RI restriction targets in the right arm and the *nin*5 deletion. λ BSilv1, *ilv*2 and *leu*3 were all *imm*⁴³⁴; λ BSilv1 and 2 formed plaques only on strains carrying *supF* and thus still carried at least the *S* mutation. λ BSleu3 and 104 were able to plaque on *supE* strains and so they had lost the *S* mutation.

Correlation of genetic markers with restriction fragments suggests that the *B. subtilis ilv-leu* gene cluster is contained in three *Eco*RI fragments of approximately 2.85, 4.8 and 6.7 kb. The entire *ilvC* gene and at least part of *ilvB* are contained in the 2.85 kb *Eco*RI fragment. Since λ BSleu3 can complement the equivalent *leuA* gene of *E. coli*, the *Eco*RI target is unlikely to be within the coding sequence of the *leuA* gene. Therefore the *Eco*RI target forming the junction of the 2.85 and 6.7 kb fragments must lie between the coding sequences of *ilvC* and *leuA*. Genes *leuA*, *C*, *B* and at least part of *D* are carried on the 6.7 kb fragment. Phages carrying DNA able to repair the *ilvB* Δ 1 deletion carried the 4.8 kb fragment; only *ilv*-transducing phages carrying this fragment complemented *E. coli* sufficiently to permit cross-feeding, which resulted in a halo of growth around the plaques.

Are spore germination genes present on the cloned DNA?

Spores of *gerF* mutants germinate poorly in a range of germinants and the *gerF* locus maps very close to the *hisA* region (Moir *et al.*, 1979). *gerF*21 is 56% co-transduced with *hisA*1 by phage SPP1 (Warburg, 1981) and *gerF*99 is 23% co-transduced with *his-11* (D. A. Walton, unpublished). Since the λ BShisA phages carried an 8.2 kb *Eco*RI insert, it was possible that the *gerF* gene could be located on it. Strains 4999 and 5176 of *B. subtilis* were transformed to *hisA*⁺

Table 4. Seeking cotransformation of *gerF*⁺, *gerE*⁺ and *gerH*⁺ in the cloned DNA

The *B. subtilis* recipients were transformed with λ BS*his*, λ BS*ilv* and λ BS*leu* phages to His⁺, Ilv⁺ or Leu⁺ and the unselected markers scored.

Donor	Recipient	Selection	Unselected phenotype	Cotransformation frequency (%)
Chromosomal DNA	4999 <i>hisA1 gerF21</i>	His ⁺	Ger ⁺	38
λ BS <i>hisA1</i> -VIII		His ⁺	Ger ⁺	< 1
Chromosomal DNA	5176 <i>hisA1 gerF99</i>	His ⁺	Ger ⁺	10
λ BS <i>hisA1</i> -VIII		His ⁺	Ger ⁺	< 1
Chromosomal DNA	4735 <i>ilvBΔ1 gerE36</i>	Ilv ⁺	Ger ⁺	24
λ BS <i>ilv2</i> , 4, 7, 11, 18, 25 and 32		Ilv ⁺	Ger ⁺	< 1
Chromosomal DNA	5185 <i>leuB16 gerH1</i>	Leu ⁺	Ger ⁺	15
λ BS <i>leu3</i> , 4, 8 and 101-5 (inclusive)		Leu ⁺	Ger ⁺	< 1
Chromosomal DNA	CU267 <i>ilvB2 leuB16</i>	Ilv ⁺	Leu ⁺	56
Chromosomal DNA		Leu ⁺	Ilv ⁺	53
λ BS <i>leu4</i>		Leu ⁺	Ilv ⁺	15
λ BS <i>leu104</i>		Ilv ⁺	Leu ⁺	12

by each of the λ BS*hisA* phages, and at least 100 transformants from each experiment tested for the cotransfer of *gerF21*⁺ or *gerF99*⁺. None was observed, although a 38% co-transformation of *gerF21*⁺ with *hisA1*⁺ was obtained when chromosomal DNA from strain 1604 was used as donor (Table 4).

gerE and *gerH* mutations map on either side of the *ilv-leu* gene cluster (Piggot *et al.*, 1981). Transducing phages carrying *ilvB Δ 1*⁺ carry the *Eco*RI fragment extending from the gene cluster in the direction of *gerE*. Seven phages were tested (Table 4) for cotransformation of *gerE*⁺ with *ilvB*⁺. None introduced *gerE*⁺ into Ilv⁺ transformants. Eight phage isolates carrying *leuB*⁺ failed to cotransform *gerH*⁺. *ilv* and *leu* markers on the cloned DNA were cotransformed at frequencies of 12–15%, which was lower than that observed with chromosomal DNA (53–56%). Cotransformation of *gerA1*⁺ with *citG4*⁺ is likewise reduced from 70% to 41–46% as compared with chromosomal DNA when λ phages or plasmids carrying these markers are used as donors (Moir & Smith, 1983; A. Moir, unpublished).

The failure to provide *gerE*⁺ or *gerH*⁺ from cloned DNA suggests that it does not extend sufficiently far to include these genes. However, because the cotransformation of markers on cloned DNA is lower than that obtained using chromosomal DNA as donor (Table 4) a failure to detect cotransformed unselected markers is not conclusive proof of their absence. Congression experiments (Moir, 1983) would be definitive. It is unlikely, however, that *gerE* is present; the 24% cotransformation of chromosomal *gerE* with *ilvB* suggests that it maps some way from *ilvB*, but it is close to *citF83* (90% cotransduced by SPP1 – Moir, 1981). None of the λ BS*ilv* phages in Table 4 transformed a *citF83* mutant to Cit⁺ (A. Moir, unpublished).

DISCUSSION

The successful isolation of phages carrying *his*, *ilv* and *leu* genes of *B. subtilis* shows that the ability of these genes cloned in λ to be weakly expressed in *E. coli* permits their selection on appropriate media. The method broadly follows the helper approach of Moir (1983) although the selection conditions used here are considerably more stringent and there are some significant differences. Most notably, the frequency of detection of phages complementing the auxotrophic markers was 3×10^{-6} – 3×10^{-7} per infected cell, whereas that for fumarase-positive clones was approximately 4×10^{-5} (Moir, 1983). This lower recovery could reflect a lower concentration of these phages in the pool but either reduced efficiency of complementation of phage functions by helper in infected cells, or poor detection of complementing plaques on a minimal growth medium, or a combination of both of these factors, could be involved.

The transducing phages obtained that carry particular *B. subtilis* genes, although separately isolated in the selection procedure, are not necessarily independent. For the *ilv-leu* gene cluster Table 3 shows that a range of isolates carrying overlapping regions were obtained. The genetical and physical data suggest that all the λ BS*his* phages isolated carry an identical 8.2 kb fragment of the *B. subtilis* chromosome. They may have originated from a single insertion event during the initial construction of the library, followed by packing and replication. Alternatively, the constraints on the size of the insert to produce viable phage may have meant that this was the only fragment carrying the particular region selected which could be inserted and that this event occurred many times.

Helper phage was essential for the initial detection of complementing plaques, as no such colony clusters were obtained when it was omitted, although it was unnecessary for complementation during later stages of isolation. Some phages would complement auxotrophic markers in strains lacking nonsense suppressors, or carrying only *supE*, despite failure of the phage to form plaques on the same strain on rich medium. It may be that during slow growth on selective medium, lysis of cells in the absence of *S* protein was sufficient to permit cycles of infection and the appearance of barely-visible plaques. The status of *W* and *E* mutations on the phages has not been established. Complementation by phages could be observed as the improved growth of small clusters of colonies of individual lysogens within an area of barely discernible lysis. The colonies were generally slow-growing and became mucoid on prolonged incubation.

Expression of cloned genes during lytic growth could have been, in part at least, from phage promoters, but expression in the lysogenic phase, the major indicator in these experiments, is more likely to be initiated from promoters in the cloned DNA. Too little is known of the organization of *his* genes in *B. subtilis* to be able to say whether the natural promoter has been cloned. For the *ilv-leu* cluster, the cloned segment spans sufficient DNA to include a promoter near the regulatory *azlA* locus upstream of *ilvB* (Mackey & Zahler, 1982); this would be consistent with the cross-feeding observed only in phages carrying the 4.8 kb (*ilvB* Δ 1⁺) fragment. Studies of azaleucine-resistant deletions (C. J. Mackey & S. A. Zahler, personal communication) indicate that the *azlA* locus and the start of the *ilvB* gene are within the 4.8 kb fragment. How expression of *ilvC* and *leu* genes from phages lacking this fragment occurs remains to be analysed. The λ BS*ilv* clones are the first to carry the regulatory region of the *ilv-leu* gene cluster, and will permit a study of the molecular biology of expression of these genes.

The observation that the *E. coli leuD* mutation was not complemented by λ BS*leu* phages was unexpected and implies that the *B. subtilis leuD* cistron is not totally contained in the cloned DNA. This would lead to the conclusion that the ability of the λ BS*leu* phages to complement defects in the *E. coli leuC* cistron would involve the cooperative function of the *E. coli leuD* and *B. subtilis leuB* gene products. Alternative explanations, also involving interaction between heterologous subunits, are possible. For example it is formally possible that *leuD* is intact in the cloned DNA but that wild-type *E. coli leuC* gene product interferes with the formation or activity of the *B. subtilis* isopropylmalate isomerase complex. This could be explored by testing the complementation of *B. subtilis leuD* mutations by the 6.7 kb *EcoRI* fragment cloned in appropriate vectors.

The 8.2 kb (4.9 MDal) insert in all the λ BS*hisA* phages isolated probably corresponds to the 4.9 MDal fragment identified in an *EcoRI* digest of *B. subtilis* chromosomal DNA when probed with a ρ 11*phisA*⁺ phage (Kawamura *et al.*, 1981). Indeed, the λ BS*hisAII* insert hybridizes to fragments of identical size when used to probe an *EcoRI* digest of *B. subtilis* chromosomal DNA (R. Morse, unpublished). The ρ 11*phisA*⁺ phage which had a 3.3 MDal insert and was thought to have suffered a deletion of *B. subtilis* DNA transduced a *B. subtilis hisA1* mutant (Kawamura *et al.*, 1979) and carried a single *Bam*HI site towards one end of the insert and two *Hind*III sites. The λ BS*hisA* phages have three *Hind*III sites so that the deletion in the ρ 11*phisA*⁺ phage may have removed at least one of these sites.

The complementation of the *E. coli hisA*, *B* and *D* genes by the λ BS*hisA* phages demonstrates that at least some *B. subtilis his* genes can function in *E. coli*. It seems unlikely that apparent complementation resulted from suppression of the *E. coli* mutations as the phages were able to

transform seven different *B. subtilis* *his* mutations. These complementation and transformation properties of the λ BS*his* phages could not have resulted from phage functions since λ^+ did not possess them (Table 2). The 8.2 kb insert therefore carries gene(s) for at least three histidine biosynthetic enzyme activities, i.e. the equivalents of *E. coli* *hisA* - N-(5'-phospho-L-ribosylformimino)-5-amino-1-(5'-phosphoribosyl)-4-imidazolecarboxamide isomerase, *hisB* - imidazole-glycerolphosphate dehydratase, and *hisD* - histidinol dehydrogenase. These phages could, therefore, be useful for the study of the genes and regulatory elements controlling histidine biosynthesis in *B. subtilis*.

The *his*, *ilv* and *leu* sequences isolated do not extend into the *ger* genes mapping nearby. The DNA clones are being used, however, as probes to detect overlapping sequences in other *B. subtilis* chromosomal libraries. The lack of direct selection for *ger* genes requires such a strategy.

The general approach described in this paper could be adopted to seek phages carrying other biosynthetic genes of *B. subtilis* which would aid comparison of the regulatory systems of *B. subtilis* with those of *E. coli* operons, the control of which is becoming clarified at the molecular level. It need not be restricted to genes in which mutations have been isolated and mapped, as an appropriate *E. coli* mutant is sufficient for the complementation approach. However, direct proof of the nature of the cloned DNA would be necessary, for example by deletion or insertion mutagenesis of the cloned DNA, followed by recombination back into the *B. subtilis* chromosome in order to test the effect of the mutation on the phenotype.

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