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*Short communication*

# The temperate *B. subtilis* phage ϕ105 genome contains at least two distinct regions encoding superinfection immunity

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**Summary.** Two different *Pst*I fragments of temperate phage ϕ105 DNA are shown to confer superinfection immunity upon *Bacillus subtilis* when inserted into the multicopy cloning vector pE194 cop-6. The 2.3 kb *Pst*I fragment I is located almost entirely within *Eco*RI fragment F and encompasses a region previously known to encode a repressor. The other fragment, *Pst*I-E (4.3 kb) maps inside the *Eco*RI-B fragment, and allows an explanation of the clear-plaque phenotype of the deletion mutant ϕ105DII:6c. The two regions can be distinguished functionally, since only the *Pst*I fragment I product interacts with a specific ϕ105 promoter-operator site.

The phenomenon of superinfection immunity whereby a resident prophage prevents its host cell from being infected productively by the same or a closely related temperate phage, is thought to involve the action of a prophage-encoded repressor, which blocks gene expression of the superinfecting DNA (Hershey and Dove 1971). The temperate *Bacillus subtilis* phage ϕ105 genome (~38 kb) is cleaved by *Eco*RI into eight fragments. One of these, the 3.2 kb *Eco*RI-F fragment (Fig. 1), has been shown to confer superinfection immunity upon *B. subtilis* when cloned on a multicopy plasmid (Cully and Garro 1980; Uhlén et al. 1981). Recently, we have mapped this function more precisely to the left 1100 bp *Eco*RI – *Hin*dIII subfragment of *Eco*RI-F (Dhaese et al. 1984).

The size and position of the deleted regions in clear-plaque mutants of ϕ105 (Flock 1977) are largely, but not entirely consistent with these cloning data. Three independent deletions extend into the left part of *Eco*RI-F, the smallest (ϕ105DI:1c) mapping between 65% and 70.3% of the genome length (Fig. 1). However, in a fourth clear-plaque deletion mutant, ϕ105DII:6C, this region is intact and a nonoverlapping region between 54.7% and 64.5% of the genome length is absent.

Another mutant, ϕ105DI:1t, lacks a region from 55.1%–64.4% of the genome length and still forms turbid plaques, indicating that most of the sequences deleted in ϕ105DII:6c are dispensable for lysogenization. In view of these somewhat puzzling results, we asked whether phage ϕ105 might contain a second region implicated in the control and repression of lytic functions.

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Phage ϕ105 DNA is cleaved by *Pst*I into at least 15 fragments, which have all been mapped (Bugaichuk et al. 1984). We ligated *Pst*I-digested ϕ105 DNA to the *Pst*I-linearized erythromycin resistance ($Em^r$) plasmid pE194cop-6 (Weisblum et al. 1979). The ligation mixture was used to transform competent cells of *B. subtilis* BR151. Plasmid-containing transformants were selected on LB plates supplemented with 10 μg/ml Em after an induction period of 1 h in the presence of 0.1 μg/ml Em (Horinouchi and Weisblum 1982). Erythromycin-resistant transformants were tested for sensitivity to wild-type ϕ105 by streaking onto LB plates previously impregnated with $5 \times 10^3$ plaque-forming units (pfu). In these tests, *B. subtilis* 168 (BGSC strain 1A1) and its ϕ105-lysogenic derivative (BGSC strain 1L11) served as sensitive and immune controls, respectively. Out of 650 $Em^r$ transformants tested, 7 colonies did not show plaque formation. Plasmid analysis showed that in six of these the 2.3 kb *Pst*I fragment I had been inserted into the vector. A representative of this class was designated pCGV14 (6.0 kb). The *Pst*I fragment I is located almost entirely within the *Eco*RI-F fragment and encompasses the 1.1 kb *Eco*RI-*Hin*dIII fragment (Fig. 1). It has been shown previously to encode a repressor function (Dhaese et al. 1984). However, one clone contained a recombinant plasmid, designated pCGV10, consisting of pE194cop-6 and the 4.3 kb *Pst*I-E fragment of ϕ105. This fragment is located within *Eco*RI-B, between 47.7% and 58.4% of the genome length (Fig. 1). To confirm that the ϕ105-immune phenotype of the latter transformant was plasmid-borne, competent *B. subtilis* BR151 were transformed with covalently closed circular DNA isolated from the primary transformant (Birnboim and Doly 1979). All of the secondary $Em^r$ transformants tested were immune to ϕ105 infection. These data suggest that ϕ105 DNA contains a second region that interferes with lytic development, when expressed from a multicopy plasmid.

Recently, we have shown that the repressor encoded by *Eco*RI fragment F interacts with a promoter-operator site located in the vicinity of its own gene (Fig. 1). We had constructed plasmids, such as pPGV10ϕ (Dhaese et al. 1984), in which this early promoter, isolated on a 650 bp *Sau*3A fragment, controls the expression of a chloramphenicol resistance ($Cm^r$) gene from *B. pumilis* (*cat-86*). To test whether or not the immunity function encoded by the *Pst*I-E fragment interacts with the same promoter-operator site, cells carrying plasmid pPGV10ϕ were transformed with

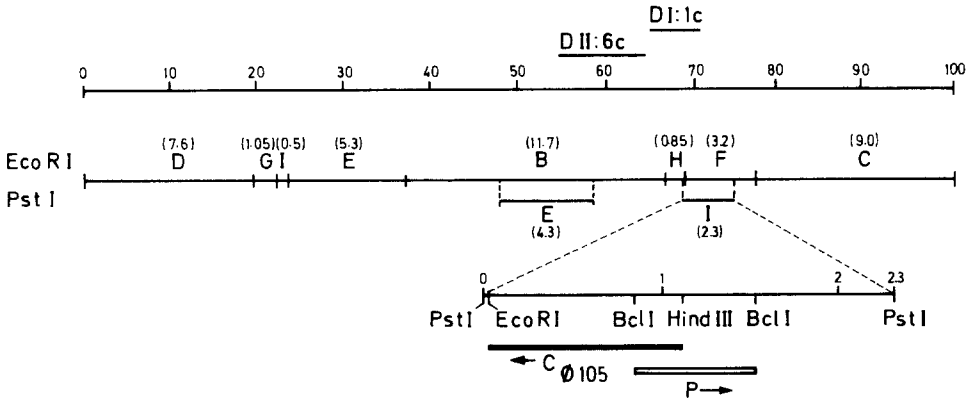


**Fig. 1.** Location of ϕ105 immunity-conferring *Pst*I fragments. The ϕ105 *Eco*RI map and fragment lengths (kb) are according to Bugaichuk et al. (1984). The positions of *Pst*I fragment E (cloned in pCGV10) and *Pst*I fragment I (cloned in pCGV14) are indicated. A more detailed map of the latter fragment shows the locations of a repressor gene (Cϕ105; *closed bar*) and a repressor-controlled promoter (P; *open bar*). The promoter had originally been cloned as a 650 bp *Sau*3A fragment (Dhaese et al. 1984). Nucleotide sequencing revealed that this was also a *Bcl*I fragment (Dhaese et al. in preparation). On *top* of the map, the position and extent of the deleted regions in two clear-plaque mutants are indicated, as determined by electron microscopy (Flock 1977)

**Table 1.** Antibiotic resistance properties of *Bacillus subtilis* BR151 double plasmid transformants[a]

| Strain [plasmid] | Colony-forming units | |
|---|---|---|
| | Em + Cm[b] | Em + Km[b] |
| BR151[pPGV10ϕ, pE194cop-6] | $2.2 \times 10^8$ | $1.8 \times 10^8$ |
| BR151[pPGV10ϕ, pCGV10] | $1.9 \times 10^8$ | $2.3 \times 10^8$ |
| BR151[pPGV10ϕ, pCGV14] | 6 | $2 \times 10^8$ |

[a] Strain BR151[pPGV10ϕ] competent cells were separately transformed with the three different erythromycin-resistant ($Em^r$) plasmids indicated; double transformants were initially selected on Em + Km (kanamycin) plates, since pPGV10ϕ contains a constitutively expressed $Km^r$ gene besides the *cat-86* gene (Dhaese et al. 1984); exponentially growing cultures of purified double transformants were next plated in the presence of the antibiotic combinations indicated

[b] Selective concentrations: Em and Km, 10 μg/ml; Cm, 15 μg/ml

each of the following plasmids: pE194cop-6, pCGV10, and pCGV14. The resulting double transformants were selected initially on Em + Km plates, followed by testing their Em + Cm resistance properties. In the absence of repressor, plasmid pPGV10ϕ confers resistance to 15 μg/ml Cm. If the insert in the pCGV plasmid encodes a repressor that blocks the promoter upstream of the *cat-86* gene in pPGV10ϕ, then a cell carrying both plasmids will have an $Em^rCm^s$ phenotype.

The results are shown in Table 1. As expected, the combination of pCGV14 and pPGV10ϕ results in the loss of the $Cm^r$ phenotype, since after direct plating of about $10^9$ cells on Em + Cm plates at 33° C only six colonies were resistant. Plasmid analysis of these $Em^rCm^r$ transformants indicated that in each case the repressor plasmid had undergone deletions in the ϕ105 insert region encoding the repressor (data not shown). On the other hand, cultures of BR151[pE194cop-6, pPGV10ϕ] and BR151[pCGV10, pPGV10ϕ] were resistant to both antibiotics, indicating that the presence of the vector plasmid or the *Pst*I fragment E does not interfere appreciably with transcription from this specific promoter.

In conclusion, our results show that the ϕ105 genome contains two distinct regions, each of which is sufficient to express superinfection immunity when present on a multicopy plasmid. One of these regions, located in *Eco*RI-F and *Pst*I fragment I, has been identified previously and extends from about 69%–72% of the genome length. Thus, we assume that this region is inactivated in the clear-plaque mutants with deletions extending beyond 70% at their right extremity (Flock 1977). We have sequenced this region and will discuss its functional organization in detail elsewhere (Dhaese et al. in preparation). Briefly, it contains a repressor gene transcribed from right to left on the conventional ϕ105 map (Fig. 1). The repressor exerts a negative control on a promoter located within the overlapping 650 bp *Bcl*I fragment (Dhaese et al. 1984), and directing transcription from left to right (Fig. 1). The second region, contained within *Pst*I fragment E, has not been reported previously. This region lies entirely in *Eco*RI fragment B, of which possibly no stable clones have been obtained in earlier studies (Cully and Garro 1980; Uhlén et al. 1981) due to its rather large size (11.7 kb).

Part of this newly identified region is deleted in the clear-plaque mutant ϕ105DII:6C (Fig. 1), indicating that it is necessary for lysogenization. Moreover, since a deletion mutant lacking a segment between 55.1% and 65% of the genome length produces turbid plaques (Flock 1977), we speculate that the right extremity of this immunity region is comprised between 54.7% and 55.1% of the genome length.

It thus appears that, although both regions are necessary for lysogenization, as shown by the deletion analysis, each on its own is sufficient to express superinfection immunity. However, the data should be interpreted with caution, since expression of fragments cloned on a multicopy plasmid might not be an accurate reflection of the prophage state. Indeed, the possibility remains that the immunity observed with the cloned *Pst*I-E fragment results from titrating out a product essential for phage replication.

Bearing these reservations in mind, it appears that the genetic organization of the ϕ105 immunity system might be similar to that of *E. coli* phage P1 (Scott 1980; Sternberg and Hoess 1983), and *Salmonella* phage P22 (Susskind and

Youderian 1983). In these phages, repression of lytic functions is also under bipartite control. One of these regions (ImmC), encoding the $c_1$ product of P1 and the $c_2$ repressor of P22, is analogous to the $\lambda$ immunity region. The other region (ImmI) encodes an antirepressor (ant), whose expression in turn is regulated by the maintenance repressor (mnt). It will be interesting to see if and how the $\phi$105 regions correspond to these well-studied Gram-negative counterparts.

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