Resistance of a *Bacillus subtilis* Mutant to a Group of Temperate Bacteriophages

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A mutant of *Bacillus subtilis* 168 was isolated which resists infection by all the group III temperate bacteriophages except SPR, while allowing full infection by phages of the other groups (I, II and IV). The mutation conferring this phenotype, *pha-3*, shows 52–54% PBS1-mediated cotransduction with the *hisA* marker, mapping therefore in the *gtaA* and *gtaB* region of the *B. subtilis* chromosome. Nevertheless, it does not affect the infection by phages sensitive to *gta* mutations.

INTRODUCTION

Very little is known about the adsorption sites of temperate bacteriophages of *Bacillus subtilis*. These phages were classified by Dean according to their serology, immunity, host range and adsorption site (Dean *et al.*, 1978). The latter criterion, although important, was poorly studied. In *B. subtilis* teichoic acid has been shown to participate in the adsorption of several lytic phages and phage $\phi 105$ (Young, 1967). Resistance to bacteriophages was exhibited by mutants blocked in the glucosylation of wall teichoic acid. Such mutants were shown to fall into three classes, *gtaA*, *gtaB* and *gtaC*, according to the gene affected. Hemphill *et al.* (1980) isolated a *B. subtilis* mutant, resistant to phage SP β c1 which was unable to adsorb phages $\phi 3T$ and SP β and verified that the resistance marker was cotransducible with the *hisA* gene. F. E. Young (cited in Zahler, 1982) obtained a $\phi 3T$ -resistant mutant which he considered to be a *gtaB* mutant based on linkage by cotransduction of the mutation with *hisA*.

We have isolated a *B. subtilis* adsorption mutant that resists infection by all the group III temperate bacteriophages ($\phi 3T$, $\rho 11$, SP β , Z, IG1, IG3 and IG4) except SPR, while retaining susceptibility to temperate phages of the other groups (I, II and IV). Based on selective bacteriophage plating efficiencies, this mutant was shown to be different from any of the *gta* mutants, although it maps in the same region as the *gtaA* and *gtaB* loci.

METHODS

The bacterial strains used are listed in Table 1. Lysates of the temperate bacteriophages $\phi 3T$ (Tucker, 1969), $\rho 11$ (Dean *et al.*, 1976), Z (Hemphill *et al.*, 1980), SP β (Warner *et al.*, 1977), SPR (Noyer-Weidner *et al.*, 1983), IG1, IG3 (Fernandes *et al.*, 1983), IG4 (Fernandes *et al.*, 1986), $\phi 105$ (Reilly, 1965), SP02 (Okubo & Romig, 1965) and SP16 (Thorne & Mele, 1974) were obtained by induction with mitomycin C (Sigma) of the lysogenic strains, as described by Rutberg *et al.* (1969). Virulent phages $\phi 29$, $\phi 25$, ϕe (Reilly, 1965) and SP01 (Okubo *et al.*, 1964) were amplified on strain CU1050.

Phages were assayed by the two-layer plating procedure (Adams, 1959) with M-agar and M-soft agar (Yehle & Doi, 1967) supplemented as described by Yasbin *et al.* (1973). Phage SP16 was assayed on PA-agar and PA-soft agar as used by Thorne (1962). Supplemented M medium (Yasbin *et al.*, 1973) was used for growing bacterial cultures.

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Table 1. *B. subtilis* strains

Strain	Genotype	Origin*
CU1050	<i>leuA8 metB5 thr-5 sup-3 attSPβ</i>	S. A. Zahler
IGCg112	<i>leuA8 metB5 thr-5 sup-3 attSPβ pha-3</i>	Spontaneous ϕ 3T resistant mutant of strain CU1050
BR151	<i>lys-3 metB10 trpC2</i>	F. E. Young
IGCg113	<i>metB10 trpC2 pha-3</i>	Congression of IGCg112 $\rightarrow$ BR151
YB886	<i>metB5 trpC2 xin-1 attSPβ</i>	R. E. Yasbin
IGCg114	<i>metB5 pha-3</i>	Congression of IGCg112 $\rightarrow$ YB886
RUB807	<i>lys-3 metB10 trpC2 gtaC20</i>	F. E. Young
RUB808	<i>lys-3 metB10 trpC2 gtaA20</i>	F. E. Young
RUB810	<i>lys-3 metB10 gtaB20</i>	F. E. Young
QB800	<i>hisA1 pha-1</i>	R. A. Dedonder
IGCg111	<i>lys-3 metB10 pha-2</i>	M. A. Santos
CU1448	<i>hisA1 thr-5 metB5 attSPβ</i>	S. A. Zahler
QB917	<i>hisA1 thr-5 trpC2</i>	R. A. Dedonder
IGCg301	<i>hisA1 cysB3 trpC2</i>	H. Paveia

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RESULTS

B. subtilis mutants resistant to infection by phage ϕ 3T were isolated by plating a ϕ 3T clear plaque mutant, ϕ 3Tc, on strain CU1050 and picking isolated colonies that survived infection. Colonies which failed to plate ϕ 3Tc, and did not produce phages upon induction with mitomycin C were selected. One representative ϕ 3T-resistant mutant was retained for further study. The mutation in this strain was called *pha-3* and it was transferred to strains BR151 and YB886 by congression.

The plating of different temperate bacteriophages on the *pha-3* mutant IGCg112 showed that phages ϕ 3T, ρ 11, SP β , Z, IG1, IG3 and IG4 were unable to infect this mutant, whereas efficiencies of plating for SPR, ϕ 105, SP02 and SP16 were respectively 1, 0.95, 0.80 and 1. These results are mean values of two experiments and determination of plating efficiencies was as described by Lencastre & Archer (1980). This mutation was thus shown to confer resistance specifically to the phages of group III with the exception of SPR, while allowing full infection by representatives of the remaining groups of temperate phages.

Adsorption studies showed that strain IGCg113 (*pha-3*) is impaired in the adsorption by phage ϕ 3T. This phage, as well as the other phages of group III except SPR, was unable to adsorb to strains lacking the *gtaB* and *gtaC* gene products, while adsorbing to the *gtaA* mutant. Among the phages tested, only phage SPR adsorbed to *pha-3* and *gtaB* mutants (Table 2).

Besides the three classes of *gta* mutations which confer resistance to some bacteriophages, two other mutations, *pha-1* and *pha-2*, were isolated which prevent adsorption by phages SP01 (Lepesant-Kejzlarová *et al.*, 1975) and SPP1 (Santos *et al.*, 1983), respectively. We verified that *pha-1* and *pha-2* mutants (strains QB800 and IGCg111, respectively) were infected with high efficiency by ϕ 3Tc; plating efficiencies were 0.95 and 0.93, respectively.

Yasbin *et al.* (1976) showed that each class of *gta* mutants can be characterized by a specific pattern of resistance to bacteriophages. In order to compare *pha-3* further with the mutations implicated in the glucosylation of cell wall teichoic acid, several phages were tested in the different bacterial mutants (Table 3). As far as the infection pattern of the *gta* mutants is concerned, our results are in agreement with those of Yasbin *et al.* (1976). All three *gta* mutations prevented infection by phages SP01 and ϕ 29. Phage ϕ 105 was unable to plate on the *gtaA* mutant and plating efficiency on the *gtaC* strain was reduced. Phage ϕ e was unable to plate on the *gtaC* mutant and phages ϕ 25 and ϕ 3Tc failed to infect either *gtaB* or *gtaC* strains. On the other hand, the newly isolated mutation did not prevent infection by all these phages, including

Table 2. Percentage adsorption efficiencies of the group III bacteriophages to *pha-3* and *gta* mutants of *B. subtilis*

To 1 ml M-broth was added 1 ml culture (1×10^8 cells) and phage to a m.o.i. ≤ 0.1 . After adsorption for 10 min at 30 °C with shaking, samples were centrifuged and supernatants assayed for unadsorbed phages. As a control, host strains were incubated as above in the absence of phage (to assay for liberation of phage SP β). Total phage added was evaluated after incubation as above, without a host strain. Results are mean values of at least two experiments. Efficiencies of adsorption lower than 18% are considered doubtful or negative because of the experimental error associated with this assay (Wilson & Takahashi, 1978).

Phage	Strain . . . (<i>gta</i> ⁺)	IGCg113 (<i>pha-3</i>)	RUB808 (<i>gtaA</i>)	RUB810 (<i>gtaB</i>)	RUB807 (<i>gtaC</i>)
ϕ 3T	98.7	5.0	96.2	7.1	0.6
ρ 11	95.2	0.0	94.3	5.9	13.1
SP β	98.1	7.9	89.2	0.02	0.0
Z	93.0	4.2	98.2	0.1	0.0
IG1	98.1	0.0	97.5	14.0	7.4
IG3	92.1	7.5	92.0	16.9	6.25
IG4	80.0	0.0	72.3	15.1	6.1
SPR	55.5	91.6	5.1	43.2	15.9

Table 3. Plating efficiencies of phages sensitive to *gta* mutations in *B. subtilis*

Plating efficiencies are mean values of three different experiments, and are given as the ratio between the number of p.f.u. obtained by plating on the different strains and the number of p.f.u. obtained by plating on strain BR151.

Phage	Strain . . . (<i>gta</i> ⁺)	IGCg113 (<i>pha-3</i>)	RUB808 (<i>gtaA</i>)	RUB810 (<i>gtaB</i>)	RUB807 (<i>gtaC</i>)
SP01	1	1.10	0(<10 ⁻⁸)	0(<10 ⁻⁸)	0(<10 ⁻⁸)
ϕ 29	1	0.90	0(<10 ⁻⁸)	0(<10 ⁻⁸)	0(<10 ⁻⁸)
ϕ 25	1	0.92	0.90	0(<10 ⁻⁸)	0(<10 ⁻⁸)
ϕ e	1	1.20	1.20	0.28	0(<10 ⁻⁸)
ϕ 105	1	0.95	0(<10 ⁻⁸)	0.45	0.03
ϕ 3Tc	1	0(<10 ⁻⁸)	0.91	0(<10 ⁻⁸)	0(<10 ⁻⁸)

Table 4. Mapping of the *pha-3* mutation relative to *hisA1* and *cysB3* markers by three-factor transduction crosses

Donor	Recipient	Selection	Recombinant class	No. of colonies scored	Implied order
IGCg114 (<i>metB5 xin-1 pha-3</i>)	IGCg301 (<i>hisA1 cysB3 trpC2</i>)	His ⁺	His ⁺ ϕ 3T ^r Cys ⁻	38	<i>cysB3 hisA1 pha-3</i>
			His ⁺ ϕ 3T ^r Cys ⁺	14	
			His ⁺ ϕ 3T ^s Cys ⁺	19	
			His ⁺ ϕ 3T ^s Cys ⁻	22	
		Cys ⁺	Cys ⁺ ϕ 3T ^r His ⁻	0	
			Cys ⁺ ϕ 3T ^r His ⁺	6	
			Cys ⁺ ϕ 3T ^s His ⁺	29	
			Cys ⁺ ϕ 3T ^s His ⁻	65	

SP01 and ϕ 29, which have an absolute requirement for glucosylated teichoic acid. The above results show that the *pha-3* mutant confers a completely different phage resistance pattern from those conferred by the *gta* mutants.

The resistance marker was mapped by PBS1-mediated transduction. Transducing lysates were prepared in strain IGCg114 (*pha-3*) by the method of Karamata & Gross (1970). Strains CU1448 and QB917 were used as recipients selecting for His⁺. The *pha-3* marker was 52–54% cotransduced with *hisA1*; no cotransduction with *thr-5* was obtained. Three-factor transduction crosses, using the same lysate and strain IGCg301 as recipient, showed the order of the markers to be *cysB3*, *hisA1*, *pha-3* (Table 4). Since there is a lack of suitable auxotrophic markers in this

region, mapping by transformation was attempted by direct selection of SP01-resistant transformants of IGCg114, with RUB808 (*gtaA*) DNA as donor. However, the number of spontaneous SP01-resistant mutants in the transforming cultures was only two- to five-fold lower than the usual number of transformants obtained. Consequently, direct selection for phage resistance upon transformation proved to be unsatisfactory.

DISCUSSION

We have isolated a ϕ 3T-resistant mutant which blocks adsorption of all phages of group III with the sole exception of SPR. The mutation *pha-3* is 52–54% cotransduced with *hisA1*. According to Young *et al.* (1969) *gtaA* and *gtaB* show, respectively, 44% and 47% cotransduction with the *hisA1* marker. The *pha-3* mutation is thus located in the same region. Nevertheless this mutation confers on the bacterial strains a completely different phage resistance pattern from those associated with all the known *gta* mutations. Further characterization of the *pha-3* mutation is currently in progress.

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