H2, a Temperate Bacteriophage Isolated from *Bacillus amyloliquefaciens* Strain H

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(Received 20 March 1987; revised 28 May 1987)

Bacillus amyloliquefaciens strain H is lysogenic for a large temperate phage we call H2. H2 has a polyhedral head 85 nm in diameter and a tail of about 17×434 nm. H2 lysogenizes *Bacillus subtilis* between the *tyrA* and *metB* genes, and gives specialized transduction of *metB* and, at lower frequencies, of *ilvD* and *ilvA*. The phage carries a thymidylate synthase gene and converts thymine auxotrophs of *B. subtilis* to prototrophy. The H2 genome is a linear DNA molecule about 129 kb in length. DNA extracted from phage particles grown in *B. subtilis* is not cut by the restriction endonucleases *Hae*III, *Fnu*4HI, *Bsp*1286I, and *Bam*HI; the latter enzyme is produced by *B. amyloliquefaciens* strain H. The prophage in lysogenic *B. subtilis* cells can be cut by these enzymes. We have isolated H2 mutants that carry the transposon Tn917, or a mutation resulting in clear-plaque morphology, or both.

INTRODUCTION

We have studied temperate bacteriophage production in several strains of *Bacillus amyloliquefaciens*, a bacterium often used in industrial fermentations (Zahler *et al.*, 1987), and have selected a phage released by strain H for detailed examination; this phage was named phage H2.

Phage H2 can carry out specialized transduction of bacterial genes close to its attachment site on the *B. subtilis* chromosome. It is of potential use in mediating gene transfer in *B. amyloliquefaciens*.

METHODS

Bacterial strains. The bacteria used in this study are listed in Table 1.

Culture conditions. Bacteria were grown in modified M Broth (MMB, Warner *et al.*, 1977). Solid medium was tryptose blood agar base (TBAB, Difco). Overlay agar for plaques was TBA (Rosenthal *et al.*, 1979). Plaque counts of H2 lysates were made in 3 ml TBA overlays with exponential-phase cells of CU3431, poured over TBAB plates containing 40 µg thymidine ml⁻¹ and incubated at 30 °C. Other incubations were done at 37 °C with suitable aeration, unless otherwise specified. Synthetic agar medium was that of Anagnostopoulos & Spizizen (1961). Thymidine (40 µg ml⁻¹) was added to all media in which strain CU3431 was grown. Selection for MLS^R was made on TBAB containing 1 µg erythromycin and 25 µg lincomycin ml⁻¹.

Preparation of phage stocks. Methods for preparing phage SPβ were described by Fink *et al.* (1981). The generalized transducing phage PBS1 was prepared by our usual methods (Ward & Zahler, 1973).

We produced satisfactory H2 lysates either by harvesting phage from agar plates (using strain CU3431 as plating bacteria), by growing the phage lytically in cultures of strain CU3431, or by inducing the lysogenic strain CU7004. The first two methods were used primarily for the production of lysates of the clear-plaque mutant H2c21. The induction of CU7004 was most satisfactory for the production of lysates of H2. The following protocol resulted in high-titre lysates suitable for DNA extraction.

Abbreviation: MLS, macrolide-lincosamide-streptogramin B.

Table 1. *Bacterial strains*

Bacterial strain*	Genotype	Notes
CU1050†	<i>leu-1050 thrA5 metB5 sup-3</i>	Zahler <i>et al.</i> (1977)
CU1064	<i>metB5</i>	Zahler <i>et al.</i> (1977)
CU1065	<i>trpC2</i>	Zahler <i>et al.</i> (1977)
CU1103	<i>citK1 leuB6 metB5 pheA2</i>	Stock strain
CU1331	<i>metB10 ilvA2</i>	Stock strain
CU1354	<i>argC4 metB5</i>	Stock strain
CU1920	<i>argA2 metB5 pheA2</i>	Stock strain
CU2958	<i>metB5 ilvD15 thyB5 ilvA2 thyA5</i>	Stock strain
CU3069†	<i>metB5</i>	Transformation: CU1065 → CU1050 (see text)
CU3431†	<i>metB5 thyB11 thyA11</i>	Double selection of CU3069 for resistance to trimethoprim (Neuhard <i>et al.</i> , 1973)
CU3617	<i>trpC2</i> (pTV1)	Stock strain
CU7004	<i>trpC2</i> (H2)	Zahler <i>et al.</i> (1987)
CU7040	<i>metB5 ilvD15 thyB5 ilvA2 thyA5</i> (H2)	H2 → CU2958
CU7048	<i>metB5</i> (H2::Tn917-1)	See text
CU7049	<i>metB5</i> (H2::Tn917-2)	See text
CU7056	<i>trpC2</i> (H2) (pTV1)	CU3617 $\xrightarrow{\text{PBSI}}$ CU7004; Selection: Cm ^R
CU7071†	<i>metB5 thyB11 thyA11</i> (H2) (H2 dmetB201)	CU7004 $\xrightarrow{\text{H2}}$ CU3431; Selection: Met ⁺
CU7075	<i>metB5 ilvD15 thyB5 ilvA2 thyA5</i> (H2) (H2 dilvA301)	CU7004 $\xrightarrow{\text{H2}}$ CU2958; Selection: Met ⁺ IlvD ⁺ IlvA ⁺ Thy ⁺
CU7077	<i>metB5 ilvD15 thyB5 ilvA2 thyA5</i> (H2) (H2 dilvD7)	CU7004 $\xrightarrow{\text{H2}}$ CU2958; Selection: Met ⁺ IlvD ⁺ Thy ⁺
CU7082	<i>metB5 ilvD15 thyB5 ilvA2 thyA5</i> (H2) (H2 dmetB205)	CU7004 $\xrightarrow{\text{H2}}$ CU7040; Selection: Met ⁺
CU8004‡	<i>B. amyloliquefaciens</i> (H2)	= strain H, Welker & Campbell (1967)
CU8060	(H2::Tn917)	H2::Tn917 conversion of CU8004; Selection: MLS ^R

* CU8000 strains are *B. amyloliquefaciens*. CU7000 strains are *B. subtilis* lysogenic for phages of *B. amyloliquefaciens*; other CU strains are *B. subtilis*. All *B. subtilis* strains carry an empty attachment site (*attSPβ*) for phage SPβ.

† These strains carry an unidentified marker named *pla-1*, originating in CU1050, that improves the ability of SPβ to form plaques on them if they are Thy⁺, and improves the ability of H2 to form plaques on them if they are Thy⁻ (see Text).

‡ Received from Bruce Carlton, University of Georgia, USA.

A sample (100 ml) of the lysogenic strain CU7004 in MMB was grown to an optical density of 25–35 Klett units (Klett–Summerson colorimeter, red filter; early exponential-phase growth). Mitomycin C was added to a final concentration of 500 ng ml⁻¹. The culture was aerated for an additional 90–120 min, and then refrigerated overnight to permit complete lysis. It was then centrifuged to remove debris.

This phage lysate was then used to infect a non-lysogenic strain (CU3431) by diluting the lysate tenfold into a growing cell culture (25–35 Klett units). The optical density was monitored at 15 min intervals. At maximum lysis, indicated by the cessation of decline in Klett value for at least 30 min, the cell debris was removed by centrifugation. NaCl (60 g l⁻¹) and polyethylene glycol (70 g l⁻¹; PEG-6000, Fisher Scientific) were added, and the lysate was mixed until the salt and PEG were in solution. This suspension was refrigerated overnight.

The PEG-precipitated bacteriophage was collected by centrifuging the PEG-treated lysate at 4000 g for 30 min in a GS3 rotor (Sorvall). It was dissolved in Tris/Mg buffer (10 mM-Tris/HCl, 10 mM-MgCl₂, pH 7.5) and layered onto a step gradient consisting of 2.5 ml each of a 1.4 and a 1.6 density (g cm⁻³) CsCl solution in a 50Ti centrifuge tube (Beckman). After centrifugation for 90–120 min at 15 °C at 100 000 g in a 50Ti Beckman rotor, the opalescent-blue phage band (between the 1.4 and 1.6 density layers) was removed with a Pasteur pipette. An equal volume of saturated CsCl solution (density 1.9) was added, and the phage was subjected to a second step gradient as described. The phage band was removed and dialysed overnight against 4 l Tris/EDTA buffer (10 mM-Tris/HCl, 1 mM-EDTA, pH 7.5).

Extraction and analysis of phage H2 DNA. H2 phage, purified through two step-gradient centrifugations and dialysed as above, was lysed with sodium dodecyl sulphate (SDS, 0.5 % final concentration) in Tris/EDTA buffer.

The DNA was extracted with phenol and then with chloroform; the extractions were repeated, and then the DNA was dialysed overnight against 4 l Tris/EDTA buffer.

The DNA was analysed with restriction endonucleases using the conditions specified by the enzyme supplier (New England Biolab or BRL). Separation of the DNA fragments, estimation of their sizes, and the production of restriction endonuclease maps of the H2 genome were done as described by Fink *et al.* (1981). Size standards of undigested SP β and λ phage DNAs, of λ phage DNA cut with *Hind*III, and of bacteriophage ϕ X174 DNA cut with *Hae*III, were run with the experimental samples.

Electron microscopy. H2 phage particles were dialysed against a 20 mM-piperazine-*N*-*N'*-bis[2-ethanesulphonic acid] (PIPES, Sigma)/10 mM-MgCl₂ buffer, pH 8.0. A sample of the phage suspension was placed on a Formvar-coated 300 mesh copper grid, and the phage preparation was stained with 1% (w/v) uranyl acetate pH 4.5 for 60 s. The stained phages were then examined with a Philips EM201 electron microscope.

RESULTS

Production of strain CU3431

We produced lysates of phage H2 by mitomycin-C-induced lysis of *B. amyloliquefaciens* strain CU8004 (= strain H), and produced a *B. subtilis* lysogen of H2 that we call CU7004 (Zahler *et al.*, 1987). Although H2 lysates produced faint clearing of overlays of strain CU1050 (our usual plating bacterium for phage SP β ; Rosenthal *et al.*, 1979) or strain CU1065, we could not detect individual plaques. Our final choice as plating host for H2, strain CU3431, was constructed by the following steps. Strain CU1050 was transformed with excess DNA extracted from strain CU1065. Selection was for Leu⁺. Among the transformants were several that had also lost (by congression) the *sup-3* marker present in CU1050; one of those, strain CU3069, had in addition lost the *thrA5* marker of CU1050. The known markers of CU3069 are *metB5 attSPβ*. It differs from other strains with (presumably) the same genotype (e.g., CU1064) in being as good a plating host for SP β as is CU1050. Like CU1050, CU3069 gives about 100 times as many SP β plaques as do CU1064 or CU1065. We assume that this is due to a genetic marker (*pla-1*) present in CU1050 that permits good plaque formation. Thus the genotype of CU3069 is considered to be *metB5 pla-1*. From strain CU3069 we isolated a thymine auxotroph by the two-step procedure of Neuhaard *et al.* (1973). This strain, CU3431, is a good plating bacterium for H2, but not for SP β ; its genotype is *metB5 thyA11 thyB11 pla-1*. We are not aware of any prophage it carries other than the defective prophage for phage PBSX (see Rutberg, 1982). We do not know why thymine auxotrophy affects the ability of these bacterial strains to produce good plaques with the two phages.

Conversion of Thy⁻ B. subtilis to Thy⁺ by H2

When turbid plaques of H2 growing on strain CU3431 were picked and purified to isolate lysogens, we found that all strains immune to the clear-plaque mutant H2c21 (see below) were also Thy⁺. The Thy⁺ phenotype was present at 50 °C as well as at lower temperatures, indicating that the *thyB* gene of *B. subtilis* (which encodes a thermosensitive thymidylate synthase) is not involved.

Bacteriophage H2 contains a thermostable thymidylate synthase gene, as do several other *Bacillus* phages (Tucker, 1969; Dean *et al.*, 1976, 1978; Fernandes *et al.*, 1983). We have labelled the H2 gene *thyH2*. This gene has been subcloned from the bacteriophage to the *Escherichia coli* plasmid vector pUC19 by complementation of an *E. coli thyA* :: Tn10 insertion.

Isolation of H2 :: Tn917

Plasmid pTV1 (Youngman *et al.*, 1983) carries a chloramphenicol-resistance gene, a temperature-sensitive replication system, and the *Streptococcus faecalis* transposon Tn917. The transposon confers resistance to erythromycin and lincomycin. This resistance phenotype is called MLS^R (macrolides, lincosamides, streptogramin B antibiotics). We moved plasmid pTV1 into the H2 lysogen CU7004 by PBS1 transduction, selecting for chloramphenicol resistance (10 µg chloramphenicol ml⁻¹). A CU7004 derivative carrying pTV1, strain CU7056, was grown at 30 °C to allow transposition of Tn917 from the plasmid to the bacterial chromosome (including the H2 prophage). Then we plated the culture at 50 °C on TBAB agar containing erythromycin

and lincomycin. The pTV1 plasmid cannot replicate at this temperature; the only cells that form colonies are those in which transposition of Tn917 to the bacterial chromosome has occurred. We pooled a few hundred such colonies, inoculated the pool into MMB, and induced the mixed population with mitomycin C. The resulting H2 lysate was used to transduce strain CU1064 *metB5* to MLS^R. Among the rare transductants, several were able to release plaque-forming H2 phage that converted lysogens to resistance to erythromycin and lincomycin. Two such transductants have been studied further. One transductant, strain CU7049 *metB5* (H2::Tn917) was used as a source of phage following mitomycin C induction. The phage was used to produce other lysogens by selecting for MLS^R. We also used phage prepared from CU7048 (probably identical to CU7049) to superinfect *B. amyloliquefaciens* strain CU8004, selecting for MLS^R. The resulting strain (labelled CU8060) released both H2 and H2::Tn917 in approximately equal quantities; presumably it is lysogenic for both phages.

Isolation of H2c21::Tn917 and H2c21

On a plate containing about 2000 turbid plaques of H2::Tn917 on CU3431, one clear plaque was seen. We purified the phage from this plaque. The progeny phage plaques were all clear. When lysates of this phage were used to transduce CU7004 to MLS^R, many transductants were found; thus the phage still carried at least part of the transposon. We have labelled it H2c21::Tn917. This phage cannot lysogenize strain CU3431 or convert it to the Thy⁺ or MLS^R phenotypes.

We used H2c21::Tn917 to convert strain CU7004, an H2 lysogen, to MLS^R. An MLS^R colony from this conversion was purified, transferred to MMB, and induced to lyse with mitomycin C. Among the progeny phage were both clear-plaque and turbid-plaque phage particles. We purified phage clones from several clear plaques. Some of these could still convert CU7004 to MLS^R; others could not. One of the clear-plaque derivatives that could not convert CU7004 to MLS^R was labelled H2c21. We assume that it resulted from a recombination event between H2c21::Tn917 and H2. H2c21 cannot lysogenize *B. subtilis* strains at detectable frequencies unless they are already lysogenic for H2 or H2::Tn917. Our evidence is compatible with the hypothesis that the *c21* mutation results in the inactivation of a H2 repressor.

Specialized transduction by H2

We first tried to determine if phage H2 could carry out generalized transduction in *B. subtilis*. Lysates of H2 induced from CU7004 could not transduce *leuB6*, *argA2* or *argC2* auxotrophs to prototrophy, but they did transduce *metB5* auxotrophs to Met⁺. This suggested that H2 could carry out specialized transduction, but not generalized transduction, and that its prophage attachment site (*attH2*) lay close to the *metB*⁺ gene of strain CU7004.

Lysates of H2 induced from strain CU7004 usually contain about 100 transducing particles for *metB*⁺ for each 10⁶ plaque-forming particles. The number of transductants is reduced tenfold or more if the recipient is lysogenic for H2. The *ilvD15* and *ilvA2* markers of *B. subtilis*, which are closely linked to *metB*, can also be transduced to prototrophy by H2 lysates from CU7004. Transduction frequencies are at least tenfold lower for *ilvD* than for *metB*, and even less frequent for *ilvA*. This suggests that the H2 prophage lies between *trpC* and *metB* on the *B. subtilis* chromosome. This position was confirmed by PBS1 mapping of the prophage (Table 2). Transduction of strain CU7004 by PBS1 phage grown in a Trp⁺ non-lysogen, with selection for Trp⁺, resulted in the loss of the H2 prophage from about one-half of the transductants. We have not been able to demonstrate specialized transduction of the linked markers *hisH2* or *tyrA2* by H2 lysates, indicating that the prophage lies between *tyrA* and *metB* (Piggot & Hoch, 1985). The gene order in the region is *trpC-hisH-tyrA-attH2-metB-ilvD-thyB-ilvA-attSPβ*.

High-frequency transducing lysates

We transduced strain CU3431 *metB5 thyA11 thyB11 pla-1*, selecting for Met⁺, with a lysate of H2 induced from strain CU7004. Among 17 Met⁺ transductants tested, 11 had become immune to the clear-plaque phage mutant H2c21 and prototrophic for thymine. We assume that some were doubly infected by H2 and by an imprecisely excised defective transducing phage H2

Table 2. Genetic location of the H2 prophage by PBS1 transduction

Transduction was achieved with strain CU1331 *attH2 metB10 ilvA2* as donor and strain CU7004 *trpC2* (H2) as recipient, with selection for Trp⁺.

Phenotype				No. of colonies
Trp	H2*	Met	Ilv	
+	—	—	—	49
+	—	—	+	7
+	—	+	+	8
+	+	—	—	2†
+	+	+	+	34
Total				100

* The presence (+) or absence (—) of the H2 prophage was determined by testing for sensitivity of the transductants to phage H2c21.

† This class was generated by double recombination events or by re-infection of phage-cured cells on the transduction plates.

Table 3. H2 specialized transducing phages from various *B. subtilis* strains

Plaque-forming particles were measured on CU3431. Transducing particles were measured on CU2958 *metB5 ilvD15 thyB5 ilvA2 thyA5*.

Donor strain and prophages	Plaque-forming particles (ml ⁻¹)	Transducing particles (ml ⁻¹)		
		MetB ⁺	IlvD ⁺ *	IlvA ⁺ **
CU7004 (H2)	1 × 10 ⁸	1 × 10 ⁴	7 × 10 ²	2 × 10 ²
CU7082 (H2) (<i>H2 dmetB205</i>)	4 × 10 ⁶	1 × 10 ⁶	<20	<20
CU7077 (H2) (<i>H2 dilvD7</i>)	2 × 10 ⁶	2 × 10 ⁵	2 × 10 ⁴	<20
CU7075 (H2) (<i>H2 dilvA301</i>)	8 × 10 ⁶	2 × 10 ⁵	1 × 10 ⁵	2 × 10 ⁴

* IlvD⁻ strains require isoleucine and valine. IlvA⁻ strains require isoleucine.

dmetB. When these 11 strains were induced with mitomycin C, three of the resulting lysates gave high-frequency transduction for *metB*⁺. The ratios of plaque-forming particles to transducing particles were between 40 : 1 and 10 : 1. None of these three strains gave a lysate that transduced *ilvD* or *ilvA*. One was kept as CU7071. Its genotype is *metB5 thyA11 thyB11 pla-1* (H2) (*H2 dmetB201*).

We constructed another strain that gave high-frequency transducing lysates for *metB*⁺ by a different protocol. We first infected strain CU2958 *metB5 ilvD15 thyB5 ilvA2 thyA5* with phage H2 to produce a lysogen we call CU7040. We transduced CU7040 to Met⁺ with an H2 lysate from strain CU7004 *trpC2* (H2). Many of the transductants produced high-frequency transducing lysates for *metB*⁺. One of those strains, CU7082, was kept for further study. We think that like CU7071, CU7082 carries two H2 prophages, one complete and one defective. Its genotype is *metB5 ilvD15 thyB5 ilvA2 thyA5* (H2) (*H2 dmetB205*).

To construct *B. subtilis* strains capable of producing high-frequency transducing lysates for *ilvD* and *ilvA*, we transduced strain CU2958 *metB5 ilvD15 thyB5 ilvA2 thyA5* with a lysate from the H2 lysogen CU7004. Selection was for Met⁺, Thy⁺, and either IlvD⁺, or IlvD⁺ and IlvA⁺. From the Met⁺, Thy⁺, IlvD⁺ selection we isolated a strain, CU7077, that gives high-frequency transducing lysates for *metB*⁺ and *ilvD*⁺. From the Met⁺, Thy⁺, IlvD⁺, IlvA⁺ selection we isolated a strain, CU7075, that gives high-frequency transducing lysates for *metB*⁺, *ilvD*⁺ and *ilvA*⁺. We named the specialized transducing phages in CU7077 and CU7075, H2 *dilvD7* and H2 *dilvA301*, respectively. Both of these strains, of course, are also lysogenic for complete H2 phage. Some characteristics of typical lysates of these strains, and of strains CU7004 (the H2 lysogen) and CU7082 (which gives high-frequency of transduction for *metB*⁺) are given in Table 3.

Table 4. *Size estimation of bacteriophage H2 DNA*

Fragment	Fragment sizes (kb)			
	<i>Sal</i> I	<i>Pst</i> I	<i>Ava</i> I	<i>Ava</i> II
A	41	32	42.5	(110)*
B	38	17.5	38	19
C	21	17	23	
D	15.5	15	13	
E	10.5	12	8	
F	5.5	6.8	3.7	
G		6.6	1.3	
H		6.1		
I		5.1		
J		4.2		
K		2.0		
L		1.1		
M		1.0		
N		0.6		
Total	131.5	127.0	129.5	(129.0)

* Estimated from average size (129 kb; this does not include the *Ava*II value).

Morphology of phage H2

We purified phage H2 from mitomycin-C-induced lysates of strain CU7004. Electron micrographs demonstrated a phage with a polyhedral (possibly icosahedral) head 85 nm in diameter (35 measurements) and a flexible tail of about 17×434 nm (20 and 16 measurements, respectively). This is consistent with the dimensions of Group III temperate phages of *B. subtilis* (SP β , ρ -11, ϕ 3T, SPR, etc.; see Dean *et al.*, 1978; Warner *et al.*, 1977; Rutberg, 1982; Zahler, 1982), although the tails of H2 are somewhat longer and thicker than those of the others.

The DNA of phage H2, and its DNA methylases

Bacteriophage H2 DNA was isolated, and digestion with several restriction enzymes revealed a linear DNA molecule with an average measured size of about 129 kb (Table 4). The size of the H2 genome is similar to those of the Group III temperate phages of *B. subtilis* (Cregg & Ito, 1979; Dean *et al.*, 1976, 1978; Fink & Zahler, 1982). We used the methods of Fink *et al.* (1981) to produce a restriction enzyme map of the H2 genome, and compared it with the map of SP β aligned so that the *Hae*III DNA methylase activities of both phages lay in the left half of the molecules (Fig. 1; see below). DNA-DNA hybridization studies comparing H2 to the Group III phages SP β , ϕ 3T and SPR show that H2 is homologous to these phages; SP β , ϕ 3T and SPR are more closely related in Southern hybridizations to each other than any of them is to H2 (data not shown).

Trautner and his coworkers (see review, Gunthert & Trautner, 1984) have shown that several of the Group III phages of *B. subtilis* encode DNA methylases that are expressed during the lytic growth cycle. Phage H2 DNA, isolated from phage grown in *B. subtilis* strain CU3431, is resistant to several restriction enzymes including *Bam*HI, *Hae*III, *Fnu*4HI and *Bsp*1286I (the target sequences of these enzymes are: GGATCC; GGCC; GCNGC; and GDGCHC respectively, where D is G, A or T and H is C, A or T). As is true of the other members of this group of bacteriophages, the modification activities are expressed only during lytic development; the H2 prophage in lysogens such as CU7004 is sensitive to these restriction endonucleases.

We have cloned each of these modification activities from H2 phage DNA and have shown that they can be expressed in *E. coli*. Experiments have been done to determine the relatedness of these modification enzymes to each other and to the modification enzymes that have been studied from other bacteriophages of the group. These results will be published elsewhere.

Fig. 1. Restriction endonuclease map of bacteriophage H2 DNA, aligned with the map of SPβ (Fink & Zahler, 1982).

DISCUSSION

Bacteriophage H2, isolated from *B. amyloliquefaciens* strain H, is related to the Group III temperate phages of *B. subtilis*. Morphologically it differs slightly from SPβ and φ3T; its tail is thicker and longer. In unpublished experiments we have found that antiserum prepared against SPβc1, a clear-plaque mutant of SPβ, cannot inactivate phage H2, and that SPβc1 does not adsorb to *B. amyloliquefaciens*. Antiserum prepared against phage H2 does not inactivate SPβ. Thus, differences in tail structure are not unexpected.

The H2 genome consists of about 129 kb of linear DNA, very similar to the 126 kb reported for phage SPβ (Fink *et al.*, 1981). When H2 lysogenizes *B. subtilis*, the prophage lies between the *tyrA* and *metB* genes on the bacterial chromosome. The prophage has thymidylate synthase activity; thymine auxotrophs of *B. subtilis* become prototrophic for thymine when they have been lysogenized by H2. When the prophage is induced with mitomycin C, many defective phage particles containing the *metB* gene are produced and can carry out specialized transduction. Smaller numbers of H2 particles carry the nearby *ilvD* and *ilvA* genes. High-frequency transducing lysates can be produced from bacteria doubly lysogenic for a defective transducing prophage and a complete H2 prophage; these lysates transduce *metB*, or *metB* and *ilvD*, or *metB*, *ilvD* and *ilvA* in *B. subtilis*. We do not know where the H2 prophage lies on the *B. amyloliquefaciens* chromosome, and we do not have mutations in that species suitable for learning if *metB* and the *ilv* genes can be transduced. Phage SPβ of *B. subtilis* can also carry out specialized transduction. Its DNA is partly homologous with that of phage H2 (Weiner and Zahler, unpublished results).

We produced some useful mutants of H2. One of them, H2::Tn917, carries the *S. faecalis* transposon Tn917. From H2::Tn917 we isolated a clear-plaque mutant, H2c21::Tn917, that can still convert H2 lysogens to antibiotic resistance. (Tn917 encodes an RNA methylase that protects Gram-positive bacteria from such antibiotics as erythromycin and lincomycin.) From such a converted strain, presumably carrying both H2 and H2c21::Tn917, we isolated the recombinant phage H2c21. This clear-plaque phage cannot lysogenize phage-sensitive cells of *B. subtilis* and does not carry Tn917.

We were able to superinfect strain CU8004, the *B. amyloliquefaciens* strain H from which H2 was isolated, with H2::Tn917. Thus we have introduced Tn917 into *B. amyloliquefaciens*, although not on a particularly useful vector. In the one case we have analysed, the antibiotic-resistant *B. amyloliquefaciens* released both H2 and H2::Tn917 when it was induced. It is possible that the phage has multiple attachment sites on the chromosomes of both species of *Bacillus*. The fact that H2c21::Tn917 can lysogenize H2 lysogens of *B. subtilis* suggests that the c21 mutation has inactivated a phage repressor but has not removed the repressor target sites.

We have isolated some thirty auxotrophic mutants of CU8004 of many different phenotypes (unpublished experiments). Unfortunately, none of them has the MetB⁻, IlvD⁻ or IlvA⁻ phenotype, so we do not know if H2 can give specialized transduction of those genes in *B. amyloliquefaciens*. Of course, the prophage site on the *B. amyloliquefaciens* chromosome may not be close to those genes; in fact, the order of genes on the *B. amyloliquefaciens* chromosome is unknown.

Phage H2 encodes proteins that protect its own DNA from certain restriction endonucleases: *Bam*HI, *Hae*III, *Fnu*4HI and *Bsp*1286I. The prophage DNA is not protected from these enzymes; the protective proteins are probably expressed only during vegetative growth of the phage. Protection from these enzymes is due to DNA methyltransferases encoded by the phage (unpublished observations). Trautner and his colleagues have similarly shown that related phages protect their DNA from *Hae*III and from *Fnu*4HI by appropriate DNA methyltransferases that are expressed only by vegetative phages (Gunthert & Trautner, 1984).

This work was supported by a grant from the Dow Chemical Company. We are grateful for useful conversations with H. E. Hemphill of Syracuse University. Bruce Carlton of the University of Georgia kindly sent us *B. amyloliquefaciens* strain H.

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