***Bacillus thuringiensis* subsp. *aizawai* generalized transducing phage ϕ HD248: restriction site map and potential for fine-structure chromosomal mapping**

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The temperate, generalized transducing phage ϕ HD248 from *Bacillus thuringiensis* subsp. *aizawai* (H-serotype 7) was shown previously to mediate transduction within subsp. *aizawai* and between subsp. *aizawai* and subsp. *kurstaki* (H-serotype 3a3b). Here it was found to have a broad host range, plating on 7 out of 14 H-serotypes tested. Cotransduction analysis using ϕ HD248 revealed two linkage groups in H-serotype 7: *bio-2-his-2-met-1-cit-1-arg-2-lys-3* and *trp-9-purC1-thi-1*. Preliminary characterization of the ϕ HD248 genome revealed a linear double-stranded DNA of 47.15 kb. A restriction site map is reported including a total of 10 sites for the enzymes *Bam*HI, *Bgl*II, *Sal*I and *Sst*I. Enzyme *Sal*I was found to have a unique cleavage site which if not located in an essential region could be used as a cloning site. The ϕ HD248 genome was also shown to lack cohesive ends and appears to have a partial circular permutation.

Keywords: *Bacillus thuringiensis*, transducing bacteriophage, restriction map, chromosomal mapping

INTRODUCTION

The *Bacillus thuringiensis* temperate phages ϕ HD67, ϕ HD130, ϕ HD228 and ϕ HD248 isolated from the subspecies *aizawai* (Inal *et al.*, 1990) have been shown to mediate both intra-subspecific (*aizawai*) and inter-subspecific (*aizawai-kurstaki*) generalized transduction (Inal *et al.*, 1992). The aim of this study was to explore the possibility of using ϕ HD248 either as a cloning vector or as a tool for mapping studies of the bacterial chromosome.

To investigate the mapping potential of ϕ HD248, cotransduction for various triple-label auxotrophs of H-serotype 7 was attempted. The proposed gene orders which resulted were then confirmed by three-factor crosses. To be useful as a vector the phage should have a broad host range. In addition it should be possible to delete non-essential regions from the phage genome, which should also possess several restriction enzyme sites. The ϕ HD248 DNA was therefore characterized by restriction endonuclease analysis to generate a physical map. The results

obtained suggest that the ϕ HD248 genome is partially circularly permuted, a feature probably due to the headful packaging mechanism; this mechanism is also the means by which generalized transduction particles result (Tye *et al.*, 1974).

METHODS

Bacterial strains and culture conditions. *B. thuringiensis* H-serotype 7 strains HD67, HD130, HD228 and HD248 were obtained from the Institute of Horticultural Research (Littlehampton, Sussex, UK). The phage indicator, the prototrophic H-serotype 7 strain HD137:12.13 was described earlier (Inal *et al.*, 1990). All strains were grown on Luria–Bertani (LB) agar (Sambrook *et al.*, 1989). Phages ϕ HD67, ϕ HD130, ϕ HD228 and ϕ HD248 were propagated on strain HD137:12.13; ϕ HD248 was plated out on different host strains as described earlier (Inal *et al.*, 1990). Double-label auxotrophic mutants were isolated by mutagenesis with MNNG (1-methyl-3-nitro-1-nitrosoguanidine) according to the methods described previously (Inal *et al.*, 1992) on the auxotrophic parental strains HD137:12.13 (*met-1*) and HD137:11.47 (*purC1*); triple-label auxotrophs were isolated similarly.

Transduction. This was carried out as described before (Inal *et al.*, 1992). For determination of cotransduction, plates containing transductants were replica-plated, using velvet, onto

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appropriately supplemented Bab minimal plates (Inal *et al.*, 1992) and incubated at 30 °C for 3 d.

Isolation of phage DNA. Phage DNA isolation (200 µg ml⁻¹) was carried out using a modification of the small-scale method of Sambrook *et al.* (1989). The phage stock at 10¹⁰ p.f.u. ml⁻¹ (20 ml) was treated with DNase I and RNase I, precipitated with PEG 8000 and then digested with proteinase K before phenol/chloroform extraction. Phage DNA in a mini dialysis chamber consisting of a 1.5 ml microfuge tube cap was then dialysed against 1000 vols TE buffer (Sambrook *et al.*, 1989) at 4 °C for 24 h with three changes of buffer.

Restriction analysis. Each reaction mixture (15 µl) containing 400 ng DNA and 4.5 units (U) of restriction enzyme (NBL) was incubated for 90 min at 37 °C (Sambrook *et al.*, 1989). All enzymes used in this study were found to be fully active in TA buffer (165 mM Tris base, 80 mM glacial acetic acid, 50 mM magnesium acetate, 320 mM potassium acetate, 2.5 mM dithiothreitol, 170 mM spermidine). Double digestions were therefore performed with both enzymes simultaneously. Electrophoresis of cleavage products was carried out in 14 × 11 cm agarose gels according to standard procedures.

For *Bam*HI partial digests a series of reactions (10 µl containing 200 ng DNA) was set up. The enzyme was serially diluted to give a concentration range from 10 to 0.08 U and the digests were incubated at 37 °C for 90 min. In this way, one or more concentrations were expected to produce partial digestion products. Digestion at the highest enzyme concentration served as the control complete digest.

RESULTS

Estimation of genome size of the transducing phages ϕ HD67, ϕ HD130, ϕ HD228 and ϕ HD248

The total sizes of the phage genomes were estimated, by *Eco*RI digestion, to be: ϕ HD67, 45 730 bp; ϕ HD130, 38 120 bp; ϕ HD228, 36 060 bp; ϕ HD248, 47 260 bp. After the construction of the restriction map (see below) the estimate for ϕ HD248 was modified to 47 150 bp. Of these four phages, ϕ HD248, which contains the largest DNA (47 150 bp), should transduce larger fragments of the bacterial chromosome. To assess the potential of ϕ HD248 as a gene transfer system it was necessary to determine its host range.

Host range of ϕ HD248

Phage ϕ HD248 grew on strains of H-serotypes 3a3b, 5a5b, 7, 8, 9, 11 and 14 but did not plate (no plaques produced) on strains of H-serotypes 1, 2, 3a, 4a4b, 6, 10 and 12 (Table 1). The broad host range of ϕ HD248, which plated on 7 out of 14 H-serotypes tested (14 out of 24 strains), thus satisfied one of the criteria for its use as a vector.

Cotransduction of H-serotype 7 strains with ϕ HD248

Cotransduction was attempted for each triple-label auxotroph available (Tables 2 and 3) using the wild-type donor phage, ϕ HD248. The percentage cotransduction values in Table 4 show that these markers form two linkage groups in H-serotype 7. In the first group, *cit-1* and *met-1* are

Table 1. Host range of ϕ HD248

H-serotype	Strain (HD)*	Lysis of test strain
1	2, 20	—
2	3, 99	—
3a	4, 16	—
3a3b	88, 102, 251, 258, 263, 270	+
4a4b	6	—
5a5b	8	+
6	9	—
7	137, 137:12.13†	+
8	12	+
9	13	+
10	146	—
11	201	+
12	542	—
14	653, 654	+

* All isolates were obtained from the Institute of Horticultural Research and originally from Dr H. T. Dulmage of the Cotton Insects Research Laboratory, US Department of Agriculture, Brownsville, TX, USA.

† HD137:12.13 is a phage-free indicator strain (Inal *et al.*, 1990).

Table 2. Genetic markers of *B. thuringiensis* used in this study

All mutations as obtained were numbered sequentially.

Marker	Phenotype (amino acid requirements)
<i>met-1</i>	Methionine
<i>bio-2</i>	Biotin
<i>arg-2 (argC1)*</i>	Arginine or citrulline
<i>cit-1 (argO1)*</i>	Arginine, ornithine or citrulline
<i>his-2</i>	Histidine
<i>lys-3</i>	Lysine
<i>purC1*</i>	Adenine or hypoxanthine
<i>thi-1</i>	Thiamin
<i>trp-9</i>	Tryptophan

* Based on the analogous phenotypes in *B. subtilis* after testing of mutants with a range of biochemical intermediates.

linked with *arg-2*, *bio-2*, *his-2* and *lys-3*. Markers that gave 0% cotransduction (data not shown) and which must therefore lie outside this group are *ala-1*, *cys-3*, *glm-3*, *gly-2*, *ile-1*, *nic-6*, *pro-1*, *pro-2* and *rib-2*. The second linkage group consists of the markers *purC1*, *thi-1* and *trp-9*. There is no linkage of markers from this group to *asp-2*, *glm-5*, *rib-4*, *rib-6*, *thy-17* or *ura-1*.

Using various recipient strains, *cit-1* and *met-1* were cotransduced at a rate of about 20%. Using isolate 20-3, the loci *his-2*, *cit-1* and *his-2*, *met-1* were cotransduced at rates of 10% and 33% respectively. The *arg-2* locus is

Table 3. Genetic markers of triple-label auxotrophic mutants isolated from strain HD137 (H-serotype 7) double-label auxotrophs by MNNG mutagenesis

Isolate	Genetic markers of isolate	Genetic markers of parental strain
19-2	<i>met-1, glm-1, bio-2</i>	(<i>met-1, glm-1</i>)*
20-1	<i>met-1, arg-2, cit-1</i>	(<i>met-1, cit-1</i>)*
20-3	<i>met-1, his-2, cit-1</i>	(<i>met-1, cit-1</i>)*
20-4	<i>met-1, lys-3, cit-1</i>	(<i>met-1, cit-1</i>)*
21-7	<i>purC1, thi-1, trp-9</i>	(<i>purC1, thi-1</i>)†
21-9	<i>purC1, thi-1, trp-A</i>	(<i>purC1, thi-1</i>)†

* The original single-label auxotrophic parental strain was HD137:12.13 (*met-1*).

† The original single-label auxotrophic parental strain was HD137:11.47 (*purC1*).

more closely linked to *cit-1* (cotransduction of 45.5–59%) than to *met-1* (cotransduction of 17.5–20%); *bio-2* is weakly linked to *met-1* (cotransduction of 12.6–14.5%) but unlinked to *cit-1*.

To construct maps for these two linkage groups, three-factor crosses involving screening of two selected and one unselected marker for some strains was carried out. For strain 20-3 it was found that 47 *cit-1, his-2* transductants also carried *met-1*, which must therefore lie between *cit-1* and *his-2*. Similarly for isolate 20-4, 43 out of 46 *met-1, lys-3* transductants also carried *cit-1*, confirming the order *met-1-cit-1-lys-3*. The remaining three transductants did not carry *cit-1* and were probably the result of double crossovers in the *met-1-lys-3* region. From these data the most likely order of these loci is: *bio-2-his-2-met-1-cit-1-arg-2-lys-3*. Cotransduction frequencies for *purC1* and *thi-1* were between 29% and 37%, indicating that they are closely linked. The *trp-9* locus (isolate 21-7) was cotransduced with *purC1* but not with *thi-1*, suggesting the order *trp-9-purC1-thi-1*.

The restriction map of the ϕ HD248 genome has a circular conformation

Initially several enzymes were used to cleave ϕ HD248 DNA. The resulting restriction profiles (Figs 1 and 2) are summarized in Table 5. The size of some of these fragments resulting from single digests was determined more accurately after double digestion (Figs 1 and 2; summarized in Table 6). The ϕ HD248 genome was thus shown to be a double-stranded DNA 47150 $\pm$ 70 bp in size. From these data, and given that all the single-digest fragments (apart from *Bam*HI-C and *Sst*I-C) were themselves cleaved at least once, it is possible to order them relative to each other and construct a restriction site map.

*Bam*HI cleaved ϕ HD248 DNA into four fragments, which were ordered by partial digestion (data not shown) as follows: *Bam*HI-A, *Bam*HI-B, *Bam*HI-C, *Bam*HI-D. With one *Bgl*II site in *Bam*HI-A and the other in *Bam*HI-D, confirming this order, so every *Bam*HI fragment must be flanked on each side, in some DNA molecules, by other

Table 4. Cotransduction of auxotrophic markers in strain HD137 (H-serotype 7) mediated by ϕ HD248

Recipient strain	Selected marker(s)	Unselected marker	Percentage cotransduction*
19-2	<i>met-1</i>	<i>bio-2</i>	14.5 (19/131)
	<i>bio-2</i>	<i>met-1</i>	12.6 (16/127)
20-1	<i>met-1</i>	<i>arg-2</i>	17.5 (75/429)
	<i>arg-2</i>	<i>met-1</i>	20.0 (61/305)
	<i>cit-1</i>	<i>arg-2</i>	58.8 (268/456)
	<i>arg-2</i>	<i>cit-1</i>	45.5 (249/547)
	<i>met-1</i>	<i>cit-1</i>	20.6 (78/378)
20-3	<i>cit-1</i>	<i>met-1</i>	20.0 (86/431)
	<i>met-1</i>	<i>his-2</i>	31.7 (120/378)
	<i>his-2</i>	<i>met-1</i>	34.9 (118/338)
	<i>cit-1</i>	<i>his-2</i>	9.5 (41/431)
	<i>his-2</i>	<i>cit-1</i>	10.9 (37/338)
	<i>cit-1, his-2</i>	<i>met-1</i>	100.0 (47/47)†
	<i>met-1, his-2</i>	<i>cit-1</i>	35.6 (57/160)†
	<i>met-1, cit-1</i>	<i>his-2</i>	20.4 (34/167)†
	<i>met-1</i>	<i>cit-1</i>	19.5 (106/543)
	<i>cit-1</i>	<i>met-1</i>	20.4 (88/432)
20-4	<i>met-1</i>	<i>lys-3</i>	13.8 (70/506)
	<i>lys-3</i>	<i>met-1</i>	16.2 (105/649)
	<i>cit-1</i>	<i>lys-3</i>	19.5 (84/431)
	<i>lys-3</i>	<i>cit-1</i>	19.7 (128/649)
	<i>cit-1, lys-3</i>	<i>met-1</i>	33.3 (34/102)†
	<i>met-1, lys-3</i>	<i>cit-1</i>	93.5 (43/46)†
	<i>met-1, cit-1</i>	<i>lys-3</i>	26.7 (23/86)†
	<i>purC1</i>	<i>thi-1</i>	37.4 (359/960)
21-7	<i>thi-1</i>	<i>purC1</i>	34.6 (167/483)
	<i>purC1</i>	<i>trp-9</i>	14.2 (93/653)
	<i>trp-9</i>	<i>purC1</i>	13.4 (58/432)
	<i>purC1</i>	<i>thi-1</i>	30.6 (354/1158)
21-9	<i>thi-1</i>	<i>purC1</i>	29.2 (288/986)

The donor strain on which ϕ HD248 was grown was a wild-type HD137 (12.13).

* (No. of cotransductants for unselected marker)/(Total no. of transductants tested).

† (No. of cotransductants for unselected marker)/(No. of cotransductants of selected markers).

full-length restriction fragments. In other words no individual *Bam*HI fragment is uniquely present at the terminus of all ϕ HD248 DNA molecules. This means that the *Bam*HI restriction sites must map in a circular array.

Amongst the restriction profiles of ϕ HD248 DNA the anomalous submolar bands also visible are labelled *Bam*HI-C1 and *Sst*I-C1. These fragments are not merely the result of incomplete cleavage because digestion with a fourfold higher concentration of the respective enzymes gave the same submolar bands. Repeated attempts to subclone these submolar fragments proved unsuccessful.

Such submolar fragments in restriction digests of phage DNA when not due to partial digestion correspond to the ends of linear DNA molecules. They derive from full-

Fig. 1. Restriction enzyme digests of ϕ HD248 DNA (0.6% gel). Lane 1, *SalI*; lane 2, *BglII* + *SalI*; lane 3, *SstI*; lane 4, *SstI* + *BglII*; lane 5, *BamHI*; lane 6, *BamHI* + *BglII*; lane 7, *BglII*; lane 8, uncut; lane 9, *SstI*; lane 10, *SstI* + *SalI*; lane 11, *BamHI*; lane 12, *BamHI* + *SalI*; lane 13, *SalI*. The asterisk in lane 10 indicates a partial fragment.

length restriction fragments and have a restriction site at one terminus while the other terminus is determined by cleavage at the *pac* site due to the DNA headful packaging system (Botstein *et al.*, 1973). Thus the 2750 bp *BamHI*-C1 band (Fig. 1, lane 5; Fig. 2, lane 3) is the '*pac*'-*BamHI* derivative of *BamHI*-C. Likewise the 4950 bp *SstI*-C1 band (Fig. 1, lanes 3 and 9) is the '*pac*'-*SstI* derivative of *SstI*-A. As the *pac* site must be the same for both the submolar fragments *BamHI*-C1 and *SstI*-C1, and by virtue of the difference in size of these fragments, 2200 bp, it was possible to map it (Fig. 3). The arrow in Fig. 3 indicates the putative direction of maturation which could generate the observed submolar fragments.

From Table 6 it is clear that all the double digests involving *BamHI* (*BglII* + *BamHI*, *SalI* + *BamHI* and *SstI* + *BamHI*) also contain the submolar *BamHI*-C1 (2750 bp). The double digests involving *SstI* (*BglII* + *SstI* and *SalI* + *SstI*) likewise have the submolar *SstI*-C1 (4950 bp) except for *SstI* + *BamHI*. This confirms the location of the *pac* site because as can be seen from the restriction map (Fig. 3) within *SstI*-C1 there is a *BamHI* site (located 2750 bp from the *pac* end) which in the *SstI* + *BamHI* digest eliminates *SstI*-C1.

SalI, which must cleave ϕ HD248 at a unique site, produces the characteristic pattern shown in Fig. 1, lanes 1 and 13 (0.6% gel) and Fig. 2, lane 5 (0.3% gel). This pattern consists of the single *SalI*-A band (30130 bp), the ladder of smaller fragments and a minor band, *SalI*-A1, of

17200 bp which is the approximate distance between *pac* and the *SalI* site. A similar profile was reported for cleavage of the *Vibrio cholerae* phage CP-T1 genome (43.5 ± 1.4 kb) with *HindIII* (Guidolin *et al.*, 1984), which also has only one restriction site. This genome was similarly reported to show a limited circular permutation and terminal redundancy. In phage CP-T1 the ladder of smaller fragments between the 30 kb *HindIII* fragment and the 13 kb minor fragment were postulated as differing from each other by the length of the terminal redundancy (which was estimated as about 4%) so that the 30 kb *HindIII* fragment was decreasing and the minor 13 kb fragment increasing in size in subsequent mature molecules.

The sites for *BamHI*, *BglII*, *SalI* and *SstI* which have been established relative to each other on the basis of the full-length fragments quoted in Table 5 have termini determined by restriction endonuclease cleavage. These sites are represented in the circular restriction map shown in Fig. 3.

ϕ HD248 DNA lacks cohesive ends

Evidence that ϕ HD248 DNA does not have cohesive ends stems from the observation that when restriction enzyme digests are heated to 65 °C for 10 min and then placed in ice/water, to break joined cohesive ends, there is no difference in the restriction profile as compared to those of non-heated digests.

Fig. 2. Restriction enzyme digests of ϕ HD248 DNA. Lanes 1 and 2, 0.6 % gel; lanes 3–6, 0.3 % gel. Lane 1, *Bam*HI; lane 2, *Bam*HI + *Sst*I; lane 3, *Bam*HI; lane 4, *Bam*HI + *Sal*I; lane 5, *Sal*I; lane 6, uncut.

Fig. 3. Restriction endonuclease cleavage map of ϕ HD248. For the construction of this map the mean size was taken as 47150 bp. As there are no known genetic markers on the DNA, the unique *Sal*I site was used as a reference. The distance of each restriction site relative to *Sal*I is given in bp.

Fig. 4. Restriction enzyme digests of λ DNA (ligated and non-ligated) and ϕ HD248 DNA ('ligated' and 'non-ligated'). Lane 1, λ *Hind*III; lane 2, ϕ HD248 *Sst*I; lane 3, ϕ HD248 *Sst*I after T4 ligase treatment; lane 4, λ *Hind*III after T4 ligase treatment.

However, cohesive ends may be very unstable and have been separated even in the absence of the disruptive conditions. To resolve this point, ϕ HD248 DNA (and λ DNA as control) was treated with T4 DNA ligase (0.01 U per μ g DNA at 12 °C for 16 h) and then subjected to restriction analysis. Phage ϕ HD248 'ligated' and 'non-ligated' DNA was digested with *Sst*I, and λ ligated and non-ligated DNA with *Hind*III (Fig. 4). In λ DNA the cohesive ends are known to occur between the 23.13 and 4.36 kb *Hind*III fragments. Clearly, when λ DNA had been ligated prior to *Hind*III digestion (lane 4), the missing 4.36 kb fragment has joined that of 23.13 kb to create a 27.49 kb fragment. In fact because of the ease of association of the λ cohesive ends even without T4 ligase (and in the absence of the disruptive conditions), in lane 1, the 4.36 kb fragment was associated with the 23.13 kb fragment (as in lane 4) although to a lesser extent. However with ϕ HD248 DNA treated with T4 ligase prior to *Sst*I cleavage (lane 3), on comparison with the 'non-ligated' ϕ HD248 (lane 2), no joined *Sst*I fragments appear. Similarly with other restriction endonucleases there was no difference in the restriction profiles of ϕ HD248 (not shown) whether or not the DNA had first been treated with T4 DNA ligase.

Confirmation that the ϕ HD248 genome is in fact linear within the phage particle and as isolated was obtained by UV irradiation (120 J m⁻²) in the presence of ethidium bromide (10 μ g ml⁻¹). This generates nicks in supercoiled DNA (Gonzalez & Carlton, 1980) but it did not alter the migration of the ϕ HD248 DNA, unlike that of the control ligated λ DNA, for which a decrease in the amount of circular DNA was observed (Inal, 1988).

Table 5. Single enzyme digestion fragments of ϕ HD248 DNA

Sizes are given in bp; those of comigrating fragments are given in parentheses. The fragments from each digest have been labelled alphabetically in order of decreasing size.

Fragment	ClaI	HindIII	EcoRI	BamHI*	SstI*	BglII*	SalI
A	8040	9860	8500	24600	27400	39900	30130
A1†	—	—	—	—	—	—	17200
B	6020	5900	6100	12700	14500	7250	
C	5380	4870	5200	7870	5250		
C1†	—	—	—	2750	4950		
D	4940	4400	4900	1980			
E	4140	4300	4500				
F	3780	4100	4250				
G	2300	3450	(2850)				
H	2000	2190	2500				
I	1790	1960	2320				
J	1750	1730	1000				
K	(1610)	1450	880				
L	1240	1280	830				
M	970	1030	580				
N	840	800					
O	810						
Total size (bp)	47220	47320	47260	47150	47150	47150	

* All fragment sizes for *Bam*HI, *Sst*I and *Bgl*II are based on the double-digest data given in Table 6.

† Bands A1 and C1 are minor bands present in submolar amounts (see text). The total sizes given, which are the summations of the fragment sizes for each enzyme, do not include the submolar bands A1 and C1.

Table 6. Double enzyme digestion fragments of ϕ HD248 DNA

Sizes are given in bp; comigrating fragments are given in parentheses. The fragments from each digest have been labelled alphabetically in order of decreasing size.

Fragment	BglII + BamHI	BglII + SstI	BglII + SalI	SalI + BamHI	SalI + SstI	SstI + BamHI
a	19200	14500	35200	14500	22150	15350
b	12700	10200	7250	12700	14500	9250
c	7870	9950	4700	10100	(5250)	7870
c1†	—	—	—	—	4950	—
d	5400	7250	—	7870	—	(5250)
d1†	2750	—	—	2750	—	—
e	1850	5250	—	1980	—	2200
e1†	—	4950	—	—	—	2750
f	130*	—	—	—	—	1980

* This fragment was detected on a 1.5% gel (not shown).

† Bands c1, d1 and e1 are the same minor bands present in submolar amounts as observed in the *Sst*I and *Bam*HI single digests (Table 5).

DISCUSSION

Further confirmation of the generalized transducing nature of ϕ HD248 was provided in the genetic analysis of H-serotype 7 which identified the linkage groups *bio-2-bis-2-met-1-cit-1-arg-2-lys-3* and *trp-9-purC1-thi-1*. Thorne (1978) described a linkage group *met-1-arg-1-arg-*

2 in H-serotype 3a and Barsomian *et al.* (1984) the linkage group *met-1-(argC1, argO1)-met-2-(pyr-1, pyrA2)* in H-serotype 7. Lecadet *et al.* (1980), using H-serotype 1 described a linkage group between *met-1* and *arg-1*. The mutant described in this study as *cit-1* responds to citrulline, ornithine or arginine (Table 2) and may be

similar to *argO* mutants of *Bacillus subtilis* (Henner & Hoch, 1980). Similarly the *arg-2* mutant which responds to arginine or citrulline (Table 2) may be analogous to the *argC* mutants of *B. subtilis* (Henner & Hoch, 1980). If this is the case the *met-1-cit-1-arg-2* linkage reported here for H-serotype 7 may be equivalent to the *met-1-(argC1, argO1)* linkage in H-serotype 7 (Barsomian *et al.*, 1984) as well as the *met-1-arg-1* linkage in H-serotype 1 (Lecadet *et al.*, 1980). Linkage groups containing *his* and *trp* have been described for H-serotypes 7 (Barsomian *et al.*, 1984) and 1 (Lecadet *et al.*, 1980). In H-serotype 7, the absence of linkage between *his-2* and *trp-9* may be due to the small size of the ϕ HD248 DNA. With respect to the *met-arg* linkage there thus appear to be similarities between H-serotypes 1 and 7, although these similarities may not extend to *his-trp*.

The inter-H-serotype (7-3a3b) transduction potential of ϕ HD248 has already been reported (Inal *et al.* 1992). The broad host range established in this study indicates that other strains can also be used in inter-H-serotype transduction using ϕ HD248.

This study has also shown that the *B. thuringiensis* transducing ϕ HD248 genome is double-stranded, and is 47150 bp in size. The genome has a circular restriction endonuclease cleavage map which has been obtained with *Bam*HI, *Bgl*II, *Sa*I and *Sst*I. It does not appear to have cohesive ends. The unique site for *Sa*I may (if it is in a non-essential region of the phage genome) be important for the use of ϕ HD248 as a vector for genes in *B. thuringiensis*.

The circular nature of the restriction map of ϕ HD248 DNA was implied from the results of *Bam*HI partial digestion and double digestion with various enzymes. The individual restriction fragments for *Bam*HI, *Sst*I and *Bgl*II are all flanked on either side by other full-length restriction fragments. No *Bam*HI, *Sst*I or *Bgl*II fragment has the same terminal position in all ϕ HD248 DNA molecules. Only if ϕ HD248 DNA consisted of a population of identical linear molecules, which would be contrary to the results presented, could a linear restriction map be proposed. The circular representation of the restriction sites does not necessarily mean that the DNA is in a physically circular conformation, and indeed evidence suggests that the ϕ HD248 genome is linear when isolated. In fact only the *Plasmaviridae* and *Corticoviridae* (both lacking tails) have circular double-stranded DNA, of approximately 13 and 9 kb respectively (Matthews, 1982).

In bacteriophages, DNA replication results in multimeric forms called concatemers. The process of maturation, whereby DNA of the size to be packaged within a phage head is derived from a concatemer, has three variations which result in the three possible arrangements of the genome of phages containing linear, double-stranded DNA.

Firstly, as typified by λ and T7, unit length molecules of identical sequence may be derived by cleavage with a sequence-specific nuclease. Such DNA with unique ends may either have short, single-stranded cohesive ends such as in the coliphages (λ , P2) or else double-stranded

terminal redundancies as in the T-odd phages (T1, T3, T7). The third possible arrangement is represented by the T-even coliphages, P22 (Jackson *et al.*, 1978a, b) and *Myxococcus* phage MX-8 (Stellwag *et al.*, 1985).

In the case of the T-even coliphages, maturation can start at a random site within the concatemer, a free end only being required. The amount packaged within the phage head being slightly larger than the genome, sequential encapsulation of more than one genome results in fully circularly permuted and terminally redundant molecules so that any part of the genome may be located at the terminus. Such genomes with a population of linear molecules with different ends give restriction profiles with some fragments containing a restriction site at one end and any particular terminus at the other. These fragments would not be present in sufficient quantity to constitute detectable bands so that all visible bands would be bounded by restriction sites and be present in equimolar quantities.

Terminally redundant molecules which are however only partially circularly permuted, as in P22 (Jackson *et al.*, 1978a, b), result from sequential encapsulation at a specific site called *pac* on the concatemer, which is only used to initiate maturation. In such phages, where only part of the genome makes up the end of the molecules, there will be some restriction fragments, flanked by a restriction site and the terminus, present in sufficient quantity to appear as visible bands in a restriction profile, albeit in submolar quantities as compared to those flanked by restriction sites at both ends. That the submolar bands *Sst*I-C1 and *Bam*HI-C1 are likely to be due to partial circular permutation of the genome with one end defined by *pac* was further shown by the inability to subclone these fragments. That no submolar *Bgl*II fragment was observed is most likely due to the limited number of encapsulations of sequential headfuls from the ϕ HD248 concatemer. We believe that this third variation, namely of partially circularly permuted and terminally redundant DNA, best describes the arrangement of the ϕ HD248 genome; the presence and size of terminal redundancies could be revealed by the action of exonuclease III or electron microscopy of homoduplex DNA.

In summary, the evidence for this arrangement of the ϕ HD248 genome is as follows. (1) The presence of submolar fragments in restriction profiles which are not the result of partial digestion and the mapping of the '*pac*' site on the ϕ HD248 restriction map which determines one end of these submolar restriction fragments. (2) The inability to subclone these submolar fragments due to the lack of a restriction site at the '*pac*' terminus. (3) Restriction of the *Vibrio cholerae* phage CP-T1 with the single-cutting *Hind*III resulting in a characteristic profile which was explained by the circular permutation of the phage genome. The very similar profile with *Sa*I, which has one site in ϕ HD248, is further strong support for the circular permutation of ϕ HD248. (4) The fact that the headful packaging mechanism which results in generalized transducing phage such as ϕ HD248 is also the mechanism resulting in a partially circularly permuted

phage genome. (5) The observation that ϕ HD248 DNA is linear and yet the restriction map is circular.

Studies on the structure of the DNA of other *B. thuringiensis* phages have revealed similar features. Homoduplex analysis (Smirnova *et al.*, 1985) of *B. thuringiensis* phage TG-2 (isolated by Aleshkin *et al.*, 1979) showed the DNA to be circularly permuted and to have end repeats. The *Bacillus* phage SP16 (Parker & Dean, 1986) was also shown to have circularly permuted and terminally redundant DNA. Similarly phage TP-21, which exists as a plasmidial prophage in *B. thuringiensis* H-serotype 3a3b strain HD-1, is believed (Ruhfel & Thorne, 1988) to be circularly permuted and terminally redundant.

Phage ϕ HD248, with a head of 30 ± 1 nm diameter (and a tail 180 ± 3 nm long) (Jones, 1984) and DNA of 47.15 kb can be regarded, for use as a transduction vehicle, as small and ideal for fine-structure mapping of the bacterial chromosome. If it is assumed that the chromosome of *B. thuringiensis* is of equivalent size to the 4188 kb of *B. subtilis* (Toda & Itaya, 1995), then, in theory, ϕ HD248 should transduce 1.1% of the *B. thuringiensis* chromosome. Examples of large and small *B. thuringiensis* phages are TP-13 and TP-18, isolated by Perlak *et al.* (1979) and Barsomian *et al.* (1984), respectively. TP-18 (DNA 54 kb; head diameter 48 nm) is small, but TP-13 (DNA 375 kb; head diameter 120 nm), which has been used to define linkage groups in H-serotype 7 (Barsomian *et al.*, 1984), would complement ϕ HD248 well, to make a complete H-serotype 7 mapping system. Although DNA cloning may now also be used for the mapping of the bacterial chromosome, transduction is still important as a natural process for gene transfer, especially for the generation of novel *B. thuringiensis* strains (Lecadet *et al.*, 1992) with a broader host range of insecticidal activity. The detailed physical characterization of ϕ HD248 presented here provides the basis for the development of this phage as a tool in the genetic analysis of *B. thuringiensis*.

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