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MP13, a Generalized Transducing Bacteriophage for *Bacillus megaterium*

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The first generalized transducing bacteriophage reported for *Bacillus megaterium* has been characterized. Optimum conditions for lysate production and transduction procedures were established so that transducing frequencies of 8×10^{-6} and higher are now possible. The phage, MP13, has a head diameter of 97 nm and a contractile tail (202 by 17 nm) and adsorbs to the periphery of the cell. MP13 was inactivated rapidly at 60°C, but not at 55°C, and was sensitive to toluene, ether, and chloroform. When centrifuged in a neutral CsCl gradient, two bands were observed, a major band of 1.490 g cm^{-3} and a minor band of 1.482 g cm^{-3} buoyant density. The major band contained only infective particles, whereas the minor band contained both infective and transducing particles. Phage DNA was resistant to several restriction endonucleases, but yielded 9 fragments with *Mbo*I, more than 34 with *Hin*dIII, and 7 with *Bst*EII. The molecular weights for the fragments from *Mbo*I-*Bst*EII double digests total 97×10^9.

Until the recent isolation of phage MP13 (31), no generalized transducing phage for *Bacillus megaterium* was available for genetic analysis of mutants in this bacterium. We reported previously that MP13 infects *B. megaterium* strains KM, ATCC13368, 899a, ATCC10778, and ATCC11561 in addition to QMB1551 (31). The phage was found to mediate generalized transduction (after inactivation by UV irradiation) at frequencies of 10^{-7} to 8×10^{-7}, which are sufficient for analysis (30), but lower than frequencies obtained with some phages active on other *Bacillus* species (23, 24, 36).

In this investigation, therefore, we defined methods for increasing the transductional frequencies of MP13 by optimizing conditions during both lysate production and transduction. Additionally, we characterized phage MP13 by studying its stability under a variety of conditions and by using electron microscopy, CsCl gradient ultracentrifugation, and restriction endonuclease cleavage of its DNA.

MATERIALS AND METHODS

Propagation and phage stability. MP13 was propagated on M medium (35), supplemented nutrient broth (22), or a minimal glucose salts medium (16), and titers were determined as described previously (31), using *B. megaterium* QMB1551 (ATCC12872) as both host and indicator. Transductions were plated on minimal medium with the Ca^{2+}, Mn^{2+}, and Fe^{2+} salts omitted. The one-step growth curve and antiserum K value were determined by the method of Adams (2). Sensitivity to heat, salts, and organic solvents were determined by the method of Carvahlo and Vary (6). Cells were infected at a multiplicity of infection (MOI) of 1 unless otherwise stated.

Transductions. Plate transductions were performed by spreading 0.1 ml of phage lysate on a selective plate, exposing the phage to 30 to 35 s of UV light at 5.2 J/m^2 per s, and then spreading 0.3 ml of recipient cells (absorbance at 660 nm [A_{660}] = 1.1) over the irradiated phage to give an MOI of 1 to 5. Recipient cells were pelleted and suspended in the same volume of minimal broth before spreading. Wild-type strain QMB1551 was used as a donor, and its mutant JV78 (*leu-4 str-3*) was used as a recipient. Antiserum against MP13 (K = 291) was prepared as described previously (13, 31) and was used in both plate and tube transductions. When tube transductions were performed, phage lysates with titers of about 5×10^9 PFU/ml were diluted 1:10 in buffer, and a 2-ml amount was exposed to 30 s of UV irradiation. The lysate was then mixed with recipient cells (A_{660} = 1.1) in a total volume of 5 ml to give an MOI of 1. After 15 min of incubation at 30°C, 0.5 ml was spread on plates with or without 0.1 ml of a 1:10 dilution of anti-MP13. Survival of the phage was approximately 10^{-5} after 30 s of UV irradiation.

Electron microscopy. The phage was prepared for electron microscopy as described previously (31) except that resuspension was in 0.1 volume of phage buffer. Measurements were calibrated by the method of Luftig (17), using catalase crystals (Ladd, Burlington, Vt.) and represent the average of 15 phage particles. Phage adsorption was carried out for 5 min to cells previously adsorbed to the grid.

Buoyant density. Cells in 1 liter of supplemented nutrient broth were infected with MP13 at an MOI of 1 and incubated with shaking at 30°C until clearing occurred after 2.5 to 3 h. The lysate was treated with DNase and RNase (final concentration, 1 μg/ml each) overnight at 4°C, clarified by centrifugation at 2,000 ×

g, and then filtered through 0.45-μm (pore diameter) membrane filters. The phage were sedimented by centrifugation at 20,000 × g for 90 min. The pellet was then suspended in 20 ml of buffer and subjected to another round of low- and high-speed centrifugations as described above. A 2-ml amount of phage suspension at approximately 10^{12} PFU/ml was added to 16 ml of CsCl (refractive index, 1.3820), and the mixture was centrifuged at 20°C for 47 h at 101,000 × g in a Beckman type 65 rotor. Fractions were collected, and the density of phage particles was calculated as described by Frampton and Mandel (12).

Buffers. The phage buffer (8) used for most phage dilutions and storage contained 7 g of Na_2HPO_4, 3 g of KH_2PO_4, 5 g of NaCl, 10 ml of 0.1 M $MgSO_4$, and 10 ml of 0.01 M $CaCl_2$ dissolved in order and adjusted to 1 liter with distilled water and to pH 7.0 with NaOH. Other phage buffers tested included SP50 buffer (3) and PBP buffer (31). SSC buffer contained 0.15 M NaCl and 15 mM sodium citrate at pH 7.0 (18). Electrophoresis buffer was 50 mM Tris base adjusted to pH 7.9 with glacial acetic acid, 2 mM sodium acetate, 0.25 mM EDTA, and 1 mM NaCl. The buffers used for enzyme digestions were those of H. Witmer, University of Illinois Circle Campus, Chicago (personal communication) and included D buffer (10 mM Tris hydrochloride, pH 7.9, 10 mM NaCl, and 0.2 mM EDTA), R2 buffer (100 mM Tris hydrochloride, pH 7.9, 50 mM NaCl, 10 mM $MgCl_2$, and 100 μg of bovine serum albumin per ml), and R3 buffer (R2 buffer except with 10 mM Tris hydrochloride). The pocket buffer contained 10 ml of electrophoresis buffer, 4 g of sucrose, and 0.025 g of bromophenol blue diluted to 50 ml with distilled water.

Restriction endonuclease cleavage and electrophoresis. Phage particles from each peak were pooled and dialyzed overnight against three 1-liter changes of buffer at 4°C. DNA was isolated and purified by the method of Marmur (18) and dissolved after the last isopropanol precipitation in D buffer. Reaction mixtures contained 80 μg of DNA in 40 μl of D buffer, 20 μl of 3× R3 buffer, and 2 μl of enzyme. All reactions were incubated for 2.5 h at 37°C except for *Bst*EII, which was incubated at 60°C for the SP18 control and at 55°C for the more heat-sensitive MP13 DNA. In the double digestions, *Mbo*I was reacted first, and then the tube was placed at 60°C before *Bst*EII was added. All reactions were in R3 buffer except those with enzymes *Eco*RI and *Bam*HI, which were done in R2 buffer. Reactions were terminated by placing the mixtures at temperatures of either 60°C or −6°C for 5 min. Controls were run under identical conditions but without enzyme. After the addition of 20 μl of pocket buffer, the DNA digests were electrophoresed with cooling on a Bio-Rad 1415 horizontal electrophoresis apparatus in a 0.3%, 0.5%, or 0.7% agarose gel (0.4 by 20 by 20 cm). Usually, 20 μl of sample (2.4 μg of DNA) was loaded into each well, and electrophoresis was carried out for approximately 9 h at 140 V. Gels were stained in 0.5 μg of ethidium bromide per ml for 2 h. Short wave UV-illuminated gels were photographed through a Wrattan 23A filter on Kodak PN55 film with a MP-3 Polaroid camera.

Chemicals. Restriction endonucleases were obtained from New England Biolabs, Beverly, Mass. SP18 DNA was the gift of H. Witmer. Agarose was from Bio-Rad, Richmond, Calif. DNase and RNase were from Sigma Chemical Co., St. Louis, Mo. CsCl was sequanal grade from Pierce Chemical Co., Rockford, Ill. All other chemicals were reagent grade.

RESULTS

Latent period. The MP13 latent period observed in the majority of one-step growth curve experiments was between 95 and 115 min. Variation in the length of the latent period seemed to be influenced by the state of cells used as inoculum. For example, cell cultures that started from heat-activated spores, rather than from cells grown overnight before inoculation, lysed as early as 75 min. The burst size showed even greater variation. Burst sizes from 240 to 882 PFU per colony-forming unit were observed under seemingly constant conditions and with MOIs of 0.1 in five experiments. The factors causing the variations were not ascertained, although similar results with other *B. megaterium* phages have been observed (J. D. van Elsas, personal communication).

Stability and morphology of MP13. Since transducing lysates should be fairly stable to facilitate genetic analysis, we monitored the viability of MP13 under a variety of conditions of temperature, propagation media, and buffers for 10 to 12 weeks. The phage very rapidly lost plaque-forming titer when stored at 4°C in M broth, supplemented nutrient broth, or phage buffer either over a drop of chloroform (99% in 2 weeks) or 1 or 2 thymol crystals (98% in 7 weeks). Even without added agents, however, the titer decreased in 12 weeks by 83 to 96% when the phages were stored in M broth, supplemented nutrient broth, SP50, or PBP buffer. MP13 was much more stable in phage buffer and in four tests lost from 0 to 39% of the plaque-forming titer over the 3-month period. Phages stored in supplemented nutrient broth plus 15% glycerol at −6°C lost only 64% viability after a year. Variation of the Ca^{2+} or Mg^{2+} ion concentration (0.001 to 0.2 mM and 0.01 to 0.1 M, respectively) of phage buffer had no effect on phage titer. Although some *Bacillus* phages have been reported to be cold sensitive (24, 36), we found that MP13 was more stable at 4°C than at 15°C or room temperature. The sensitivity of MP13 to heat was also tested, and the phage was stable at 55°C for 30 min, but lost 60% of its plaque-forming titer after 30 min at 60°C.

Since MP13 was sensitive to prolonged exposure to chloroform at 4°C, we measured the sensitivity of phage to 17% (vol/vol) chloroform, to the solvents toluene and ether at 30°C for 30 min, and to increased salt concentration by the methods of Carvahlo and Vary (6). MP13 exhibited no decrease in titer on plates with 60 mM KCl, and only 8% and 12% loss of titer on 60 mM $CsSO_4$ and $MgSO_4$, respectively. However,

30-min exposures to each of the organic solvents caused between 86-90% loss of phage viability.

MP13 formed clear to turbid plaques of approximately 1 to 1.5 mm with a ring of cells and a halo on M or supplemented nutrient broth soft agar overlays. Sometimes a larger, outer, very turbid halo was also observed. A few small plaques (0.2 to 0.5 mm) were always present, but when either large or small plaques were picked to new soft agar, they produced both plaque sizes with small plaques always in the minority (approximately 20%). Stable lysogenic cells have not been detected, although MP13 can be trapped within spores (unpublished data).

The phage morphology and sites of adsorption are shown in Fig. 1. MP13 has a contractile tail with curly tail fibers which surround a large base plate (Fig. 1a and b). Isolated upturned base plates made up of subunits with shared six-fold symmetry are also shown in Fig. 1a and b. There are at least 40 striations in the tail, and these were sometimes rearranged longitudinally in the contracted tail sheath. The large triangular facet observed frequently upon negative staining sug-

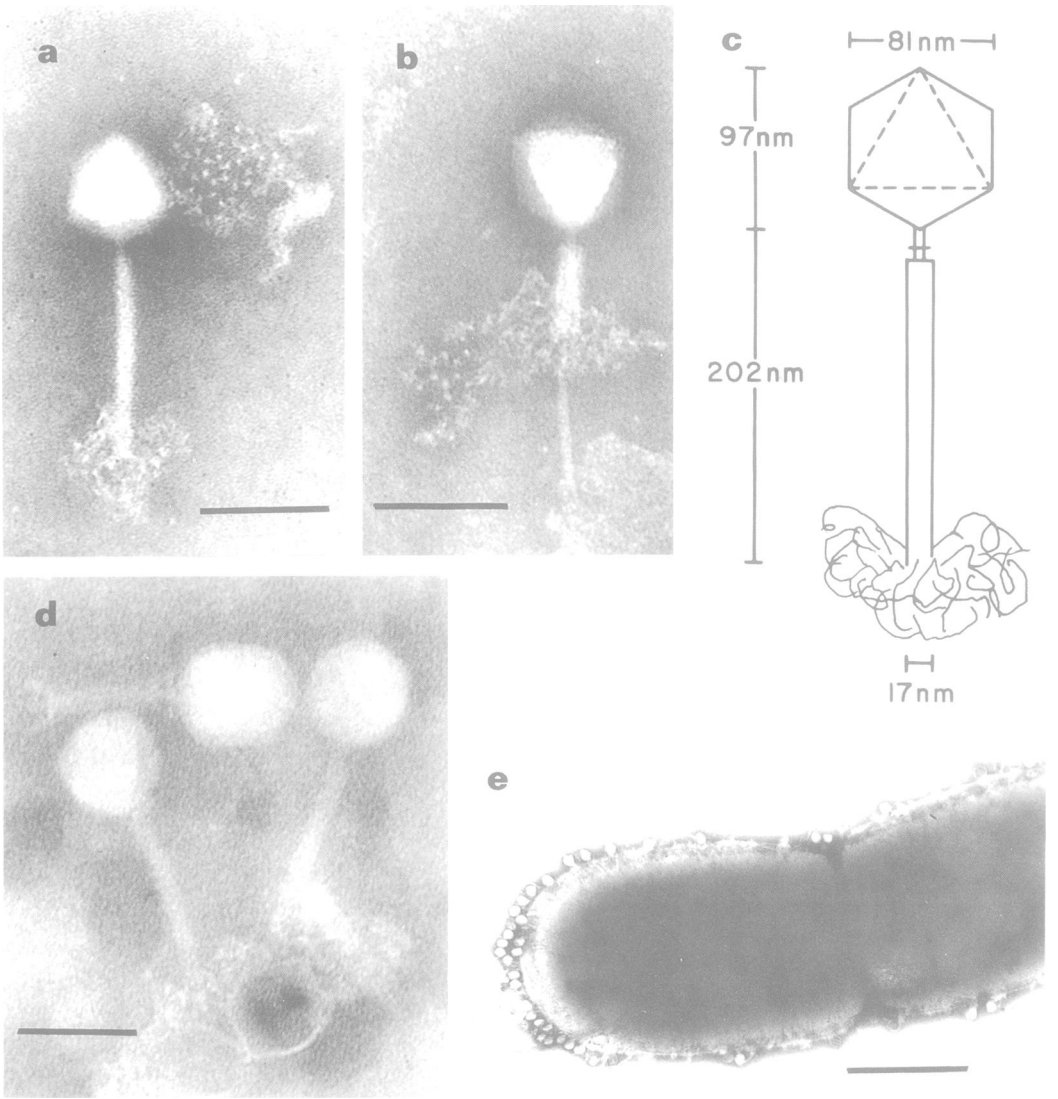

FIG. 1. Morphology of MP13. Phages were stained with 2% phosphotungstic acid, pH 7.0. (a) MP13 uncontracted. Note also the upturned base plate. (b) MP13 with contracted tail sheath. (c) Dimensions of MP13. (d) MP13 and T4 (middle). (e) *B. megaterium* QMB1551 after 5 min of adsorption of MP13. Bars represent 100 nm in a, b, and d; and 1 μm in e.

gests octahedral symmetry, according to Bradley (4). The dimensions of MP13 are summarized in Fig. 1c, and it is evident from Fig. 1d that MP13 is just slightly smaller than coliphage T4, which has a head measuring 85 by 115 nm (1). Adsorption of MP13 was found to be at the periphery of the cell, with more phages accumulating at the ends of the cells (Fig. 1e).

Infectivity and transduction by MP13 from CsCl gradients. Phage were centrifuged in CsCl gradients as described in the text (Fig. 2). Two peaks were observed, a major peak (80%) with a density of 1.490 g cm^{-3} and a lighter peak (20%) with a density of 1.482 g cm^{-3} density. Peak fractions were pooled and dialyzed overnight at 4°C against several changes of phage buffer. No differences in morphology were obvious when phages from both peaks were examined by electron microscopy. In two different gradient preparations, the major peak gave titers of 2.4×10^{10} and 2×10^{11} PFU/ml, 2 to 10 times higher than those of the minor peak (1.3×10^{10} and 2.1×10^{10} PFU/ml). When phage from both peaks were tested for ability to transduce strain JV78 to leucine prototrophy, those in the major peak produced no detectable prototrophic colonies (frequency, $<2 \times 10^{-9}$), whereas those in the minor peak produced transductants at frequencies of 5×10^{-5} and 1.6×10^{-7} transductants per PFU. The prototroph colonies formed were always very small, but when transferred to fresh minimal medium, they grew to normal size, indicating that they were not abortive transductants. When phages from the major peak of the CsCl gradient were propagated and centrifuged in CsCl as before, two peaks of similar density and proportion to those shown in Fig. 2 were again observed. Phage particles from each peak still produced two plaque sizes.

When MP13 lysates were irradiated in buffer at 5.2 J/m^2 per s, two populations were again detected (data not shown): 99% of the phage were inactivated rapidly within 30 s, and then the remaining 1% was inactivated at a slower rate. Both populations exhibited one-hit inactivation kinetics. The more resistant population gradually disappeared in inactivation curves of older lysates upon storage at 4°C, as did the transducing ability of those lysates.

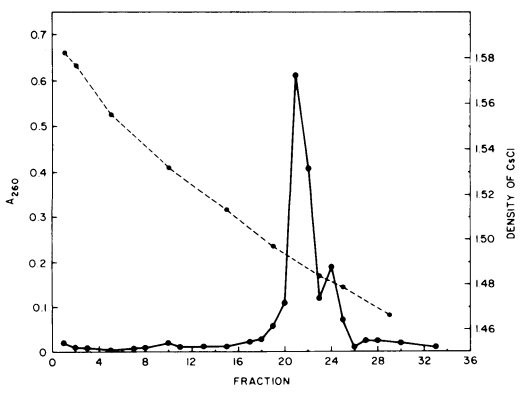

FIG. 2. Buoyant density centrifugation of MP13 particles in CsCl.

Restriction endonuclease analysis. Phage particles of two densities were observed, but we have found that the densities of DNA in both bands were the same (M. Mandel, D. Robberson, A. Ansevin, D. Vizard, E. Frampton, and P. Vary, manuscript in preparation). Phage particles therefore might contain different amounts of DNA which might be detected as differences in restriction fragments. DNA purified from phage particles in each band was digested with a variety of restriction endonucleases.

Other findings indicate that MP13 DNA was not cleaved by *Xho*I, *Sal*I, *Pvu*I, or *Pvu*II but could be cleaved more frequently with *Hin*dIII, *Bst*EII, and *Mbo*I (H. Witmer, personal communication). We found that the phage DNA from both peaks was also resistant to *Eco*RI and *Eco*RII, *Bam*HI, *Pst*I, and *Bgl*I. Fragments resulting from digestion with *Hin*dIII are shown in Fig. 3. At least 34 fragments could be detected and consistently showed intense and light bands. Fragments generated from *Mbo*I, *Bst*EII, and *Mbo*I-*Bst*EII double digestions electrophoresed in 0.7% agarose are shown in Fig. 4. The molecular weights of the fragments (Table 1) were calculated by measuring photographic enlargements and comparing them with a SP18 *Bst*EII-generated standard. *Bst*EII fragments A, B, C, D, and E were resolved on 0.3% and 0.5% gels. *Mbo*I digestion resulted in nine fragments plus a barely visible fragment (e) of 6.8×10^9 daltons consistently present. This band may be a partial digestion band of fragments g and h. The double digestion resulted in the appearance of new bands of molecular weights 8.3×10^9, 6.2×10^9, and 2.2×10^9 and the disappearance of *Bst*EII fragments of A, D, and E and *Mbo*I fragments a, b, and h. No differences between the digestion fragments produced from either the large or small CsCl bands phage DNA could be detected.

Optimum conditions for transduction. An effort was made to increase the transduction frequencies for MP13 by varying the conditions for both producing transducing particles and for transductions. When conditions for lysate production were varied, the resulting lysates were used to transduce JV78 to leucine prototrophy under constant conditions as follows: 30 s of UV inactivation of phages spread on minimal medium plates, subsequent spreading of recipient cells (A_{660} = 1.1) to give an MOI of 1, and incubation at 30°C for 24 to 48 h. Appropriate

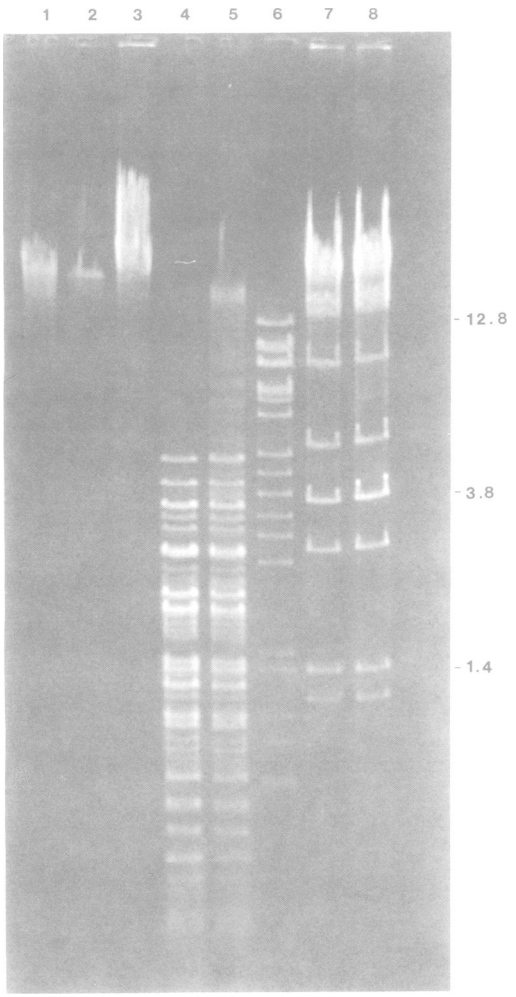

FIG. 3. *Hin*dIII and *Mbo*I digestion of DNA from both types of MP13 phage particles. Electrophoresis of digests was done in 0.7% agarose. Lanes 1, 2, and 3 are undigested DNA of SP18 phage DNA, MP13 from the large CsCl band, and MP13 from the small band, respectively. Lane 4 is *Hin*dIII-digested large-band DNA; lane 5 is *Hin*dIII-digested small-band DNA; and lane 6 is *Bst*EII-digested SP18 phage standard. Lanes 7 and 8 are *Mbo*I-digested large and small bands, respectively.

phage and recipient controls were always included. Variables tested during lysate production included medium (M, supplemented nutrient broth, and minimal), temperature (24°C, 30°C, and 37°C), pH (6.0 to 8.0), MOI (0.1 to 100), and amount of aeration during growth. Phage propagation in supplemented nutrient broth or minimal medium at either 30°C or 37°C was optimal, and either pH 8 or reduced aeration produced lysates with transduction frequencies up to 5.3 $\times$ 10^{-6} per PFU. Titers obtained at pH 8, however, were low, and the phage lost viability quickly when stored at that pH. Since lysates that were shaken at 100 rpm until lysis was complete (about 6 h at the lowered aeration) in supplemented nutrient broth at pH 7 gave titers of 9.8×10^9 to 3.1×10^{10} PFU/ml and transduction frequencies of 3×10^{-6} to 5×10^{-6} per PFU, this procedure was adopted as the standard method for lysate preparation. Lysates produced by the soft agar overlay method in supplemented nutrient broth agar at pH 7 and 8 gave slightly lower transduction frequencies.

To increase transduction frequencies further, conditions of lysate production were kept constant, and transduction conditions were varied. Besides the same variables tested above, other conditions tested were the stage of growth of cells at the time of infection (A_{660} = 0.5 to 1.2) and spores, UV exposure (15 to 45 s at 5.2 J/m^2 per s), incubation temperature (4°C to 45°C), presence or absence of MP13 antiserum on selective plates, and plating media. Media tested included minimal, minimal without Ca^{2+}, Mn^{2+},

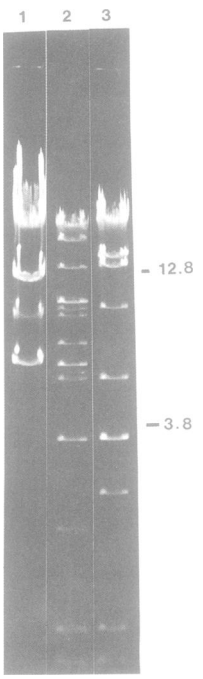

FIG. 4. Restriction endonuclease cleavage of DNA from phage in the small CsCl band. (1) *Bst*EII digest fragments; (2) fragments from double digest with *Mbo*I and *Bst*EII; (3) *Mbo*I digest fragments. Phage SP18 DNA digested with *Bst*EII was used as a standard. Electrophoresis of digests was in 0.7% agarose as described in the text.

TABLE 1. Molecular weights of restriction fragments of phage DNA from both large and small CsCl bands

DNA fragment mol wt (10^6) for following band[a]:											
*Hin*dIII			*Mbo*I			*Mbo*I-*Bst*II			*Bst*EII		
Fragment	Large	Small	Fragment	Large	Small	Fragment	Large	Small	Fragment	Large	Small
A	4.6	4.6	a	25[b]	26[b]				A	22[b]	21.5[b]
B	4.0	4.0	b	16.8	17.5	B	17.6	17.8	B	18	17.7
b1	3.8	3.8	c	14.0	14.5	C/b	16.5	16.5	C	16	16.6
b2	3.6	3.7	d	8.6	8.6	c	14.0	14.0	D	11.5	11.5
C	3.5	3.5	e	6.8	6.7		**8.8**[c]	**8.9**	E	10.5	10.7
c1	3.2	3.3	f	4.95	5.0	d	8.3	8.3	F	7.5	7.5
c2	3.0	3.1	g	3.5	3.6	F	7.75	7.6	G	5.2	5.2
D	2.7	2.7	h	2.7	2.7	e	6.7	6.7			
E	2.6	2.6	i	1.35	1.30		**6.2**	**6.0**			
e1	2.4	2.45	j	1.17	1.12	G	5.3	5.35			
e2	2.25	2.3				e	4.6	4.7			
e3	2.2	2.2				f	3.65	3.65			
F	2.15	2.1					**2.2**	**2.2**			
G	1.9	1.95				h	1.35	1.3			
g1	1.82	1.85				i	1.15	1.1			
g2	1.71	1.70									
g3	1.6	1.67									
g4	1.6	1.62									
H	1.4	1.45									
I	1.32	1.37									
J	1.20	1.21									
j1	1.10	1.18									
K	1.10	1.04									
L	0.96	0.98									
l1	0.88	0.90									
l2	0.82	0.84									
l3	0.80	0.80									
l4		0.78									
M	0.71	0.72									
N	0.60	0.61									
O	0.53	0.54									
P	0.44	0.45									
Total	60.47	62.01		84.87	87.02		97.4	97.4		90.7	90.5

[a] The more intense bands are designated by capital letters.
[b] Estimate, since the agarose gels were not linear at this size.
[c] New bands not found in either of the single digests are shown in boldface type.

or Fe^{2+}, and several media reported previously, such as Min1, Min3, Min3C, Min7, and Min10 (23, 24, 25, 26) containing various amino acids, casein, or broth supplements. No large differences were observed among several supplemented minimal media tested except that some supplements allowed too much growth of the recipient leucine auxotroph. The highest transduction frequencies (3×10^{-6} per PFU) were observed in cells infected at an MOI of 1 in late logarithmic phase with phages inactivated at from 20 to 40 s, plated on minimal media at pH 6.5, and incubated at 30°C. Antiserum enhanced the recovery of transductants in broth, but the transduction frequencies observed in such transductions, using both UV irradiation and antiserum, were about the same as plate transductions with UV irradiation alone. By using the optimum conditions for both lysate production and transductions, frequencies of 8×10^{-6} per PFU and higher could be obtained.

DISCUSSION

MP13 has several characteristics which are advantageous for genetic analysis. First, the phage is larger than SP10 (26), making it a useful transducing phage for mapping the *B. megaterium* chromosome. Second, since it adsorbs to the cell and not to flagella, motile recipients are not required for transduction. Third, it can be maintained at 4°C in phage buffer for at least 3 months or at −6°C in supplemented nutrient broth plus 15% glycerol for over 1 year, provided that chloroform or thymol are not added to the lysate. Transduction frequencies of MP13 can be increased by optimizing the conditions of

both lysate production and transduction procedures. The proportion of transducing particles was shown to increase in lysates in which there was higher than optimum pH or lowered aeration. Frequencies of 8×10^{-6} transductants per PFU for leucine prototrophy, with occasional frequencies of 10^{-5}, could be obtained when optimum conditions for both lysate production and transduction were used. This represents up to an 80-fold increase over the frequencies first reported (30) and compares favorably with the 10^{-4} to 10^{-7} frequencies obtained for *B. subtilis* phage SP10 (23).

An unexpected result was the presence of two bands after centrifugation in CsCl. The lighter minor band contained most, if not all, of the transducing particles and many infective particles. We found recently that the density of MP13 DNA from both major and minor bands is identical; that is, 1.736 g cm^{-3}. This DNA has a melting point of only 65.2°C and a substituted hydroxymethyl uracil replacing 20% of the thymine (Mandel et al., manuscript in preparation). One possible explanation for the lighter minor band might be that a fairly frequent event occurs in which less than a headful of phage DNA is packaged, thus increasing the protein-to-DNA ratio. Aberrant packaging could also result in bacterial DNA being packaged occasionally, producing the transducing particles observed in the lighter band. Phage from either small or large plaques produce both sizes of plaques when picked to new soft agar overlays. There are always approximately 20% small plaques formed, no matter which phage source was used. Large plaques could be formed when a DNA of greater than one genome size is packaged, perhaps because of a physical or dosage effect. Small plaques, containing a full genome, but less than a headfull of DNA, could then be expected to also produce both sizes of plaques if concatemers were formed before packaging and the same packaging errors occurred. Humphreys and Trautner have recently shown that such a packaging error is present in *B. subtilis* phage SPP1, which frequently makes a 200-base-pair error during packaging (15).

If MP13 DNA were packaged by cleavage at unique sites as in lambda and the T-odd phages (15), and if the lighter particles contained less DNA, one or more restriction endonucleases fragments should be missing from the ends of the small band phage DNA. This property was not observed since the digests were identical. In addition, *Hin*dIII and *Mbo*I digests of DNA from both types of phage particles consistently gave patterns in which minor fragments were present in nonstoichiometric amounts. This is frequently observed in digests of circularly permuted DNA (15). If MP13 does contain circularly permuted DNA, then this would explain why unique end fragments were not observed. All restriction endonucleases which cut MP13 DNA contain a thymine in their recognition sequences. Another explanation for the minor fragments could be that the thymine is sometimes replaced by the substituted hydroxymethyl uracil and becomes resistant to cleavage, generating the minor fragments or "partials" observed. The lack of other restriction endonucleases able to cleave MP13 DNA has hampered attempts to construct a restriction fragment map of the phage chromosome and to determine whether partial digestion is occurring.

The presence of different lengths of DNA in the phage particles should have been fairly easy to determine by molecular weight measurements, but attempts to obtain a molecular weight for MP13 DNA by sedimentation velocity have so far failed because of unusual properties of the phage DNA which are currently under investigation (unpublished data). Electron microscopic measurements of DNA length have similarly met with difficulties (M. Mandel, personal communication). Therefore, it is not clear at this time whether the lengths of the DNA in the two peaks are different. Estimates of the molecular weight of phage DNA based on head volume calculations (ignoring adjustments for unusual bases and differences in guanine plus cytosine content) give an upper limit for MP13 DNA of about 100×10^6 g/mol, approximately 13% less than the value for unglucosylated phage T4c DNA, which is 114.9×10^6 g/mol (7).

The totals of the fragment molecular weights are shown in Table 1. *Hin*dIII fragment totals were low since many small fragments were lost from the gel. The totals for *Mbo*I and *Bst*EII single digests were probably low because fragments a and A were underestimated. The value of 97×10^9 found for the double digests was in good agreement with the molecular weight estimate based on head volume.

The characteristics reported here for MP13 are similar to those of some other *Bacillus* phages. For example, sensitivity to chloroform has also been observed for *B. megaterium* phages CS-1 and ϕT (9, 14), but the reason for this sensitivity was not investigated. The heat sensitivity to 60°C but not 55°C is similar to that for *B. megaterium* phage MP7 (6). MP13 is also similar in certain respects to the *B. subtilis* transducing phage SP15. SP15 is a contractile-tailed phage which has a long latent period and sometimes exhibits major and minor bands in CsCl (Witmer, personal communication). The DNA from SP15, with a density of 1.761 g cm^{-3} and a T_m of 61.5°C, has been reported to contain an unusual pyrimidine replacing part of the thymine (19). Several other *Bacillus* phages also

contain unusual pyrimidines, including PBS1, SP8, SP10, SP5C, SP82, and φe (20).

Recently, cell fusion (11) and polyethylene glycol-mediated plasmid DNA transformation (5, 32) have been reported in *B. megaterium*. Neither of these methods has yet been effective for genetic analysis of chromosomal markers. However, MP13 transduction has now been successfully used for mapping studies of the leucine region of the *B. megaterium* chromosome (13). The availability of a well-characterized generalized transducing phage should now make possible the analysis of many interesting mutants (10, 21, 27, 33), including the mapping of several mutants blocked in an early step of spore germination (28, 29, 34).

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