Discovery, Purification, and Characterization of a Temperate Transducing Bacteriophage for *Bordetella avium*

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We discovered and characterized a temperate transducing bacteriophage (Ba1) for the avian respiratory pathogen *Bordetella avium*. Ba1 was initially identified along with one other phage (Ba2) following screening of four strains of *B. avium* for lysogeny. Of the two phage, only Ba1 showed the ability to transduce via an allelic replacement mechanism and was studied further. With regard to host range, Ba1 grew on six of nine clinical isolates of *B. avium* but failed to grow on any tested strains of *Bordetella bronchiseptica*, *Bordetella hinzii*, *Bordetella pertussis*, or *Bordetella parapertussis*. Ba1 was purified by CsCl gradient centrifugation and was found to have an icosahedral head that contained a linear genome of approximately 46.5 kb (contour length) of double-stranded DNA and a contractile, sheathed tail. Ba1 readily lysogenized our laboratory *B. avium* strain (197N), and the prophage state was stable for at least 25 generations in the absence of external infection. DNA hybridization studies indicated the prophage was integrated at a preferred site on both the host and phage replicons. Ba1 transduced five distinctly different insertion mutations, suggesting that transduction was generalized. Transduction frequencies ranged from approximately 2×10^{-7} to 1×10^{-8} transductants/PFU depending upon the marker being transduced. UV irradiation of transducing lysates markedly improved transduction frequency and reduced the number of transductants that were lysogenized during the transduction process. Ba1 may prove to be a useful genetic tool for studying *B. avium* virulence factors.

The study of temperate phage that infect pathogenic bacteria has provided numerous insights into the virulence of the host bacterium. The role of a temperate phage in virulence can be quite direct, in that some temperate phage carry toxin genes required for the host bacterium (lysogen) to cause disease (5, 26, 38, 39). In other cases, lysogeny affects virulence in more subtle ways that have to do with cell surface alterations (2, 3, 28, 34). Mutations to phage resistance often affect virulence properties by altering the cell surface, rendering the bacterium impaired not only in its ability to interact with phage but also with the host (8, 9, 30, 35). In addition to providing insights into the pathogenic process, temperate phage are often found to be generalized transducing phage (31). Such transducing phage provide valuable tools to characterize pathogenic bacteria genetically, especially in those pathogens where the means for genetic exchange via other mechanisms are limited (17, 18).

Bordetella avium causes bordetellosis, an upper respiratory tract disease in birds. Commercially raised turkeys are particularly susceptible (33). The disease involves colonization of the trachea, resulting in the death of ciliated tracheal cells. Experimentally infected birds normally recover after several weeks (36), but naturally infected birds are subject to a variety of secondary infections which cause severe economic losses in all poultry-producing regions of the world (33). The virulence-associated factors of *B. avium* are not well defined.

In this communication, we report the discovery of a *B. avium* temperate transducing phage (Ba1). We determined the host range of the phage and its biochemical and physical properties. Also, we characterized the nature of the Ba1 prophage state. In

addition, we present evidence that transduction is generalized, having successfully transduced five randomly chosen insertion mutations. Finally, we identify procedures to increase transduction frequency and reduce lysogenization of transductants.

MATERIALS AND METHODS

Strains and growth conditions. Bacteria and bacteriophage used in this study are listed in Table 1. *B. avium* and *Bordetella hinzii* broth cultures were grown at 37°C with shaking in brain heart infusion (BHI) medium (Difco) as described previously (37). BHI agar plates consisted of BHI broth with 1.5% agar added. Soft agar for preparing top-agar lysates contained 0.7% agar. Bacteria to be phage infected were grown overnight to stationary phase. The preparation of 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal) indicator agar and minimal medium for *B. avium* has been previously described (37). *Bordetella pertussis* and *Bordetella parapertussis* were maintained on Bordet-Gengou agar (BBL Microbiology Systems, Cockeysville, Md.) with 15% sheep's blood. Phage sensitivity spot tests with these two strains employed a minimal medium (Stainer-Scholte) described by Hewlett and Wolff (16).

Preparation of Ba1 lysates for phage purification. A portion of an overnight culture of *B. avium* 197N (typically 0.2 ml containing approximately 10^9 CFU) was mixed with a portion of a Ba1 lysate in a 15-ml test tube to produce a ratio of approximately 10^{-4} PFU: 1 CFU in a total volume of 0.3 ml. After a 20-min adsorption at 37°C, equal volumes (3 ml each) of soft agar and BHI broth were added, and the contents were mixed and poured onto a freshly made BHI agar plate. After a 10- to 11-h incubation period at 37°C, lysates were harvested by scraping the soft agar layer into a 40-ml centrifuge tube, adding approximately 0.2 ml of CHCl_3 , and vigorously mixing the contents on a vortex mixer for 30 s. Following a 20-min incubation at room temperature, agar and cell debris were removed by centrifugation at $7,800 \times g$ for 10 min. The resulting supernatant, referred to as crude lysate, had an average titer of approximately 5×10^9 PFU/ml (titration was performed essentially as for lysate preparation except that 3 ml of soft agar overlay was employed with serial dilutions of the lysate in 0.15 N NaCl). The addition of either MgCl_2 or CaCl_2 during adsorption had no effect upon the resulting titer. For further purification, phage were isolated from the crude lysate by centrifugation for 3 h at $31,200 \times g$. The resulting phage pellet was resuspended in 10 mM Tris-HCl-5 mM MgCl_2 (λ -dil [10]), and the phage was isolated on a CsCl step gradient.

Phage isolation and nucleic acid extraction and manipulation. An eight-step 17-ml CsCl gradient (41) subjected to centrifugation for 20 h at $116,000 \times g$ in a Beckman SW28 rotor was used to estimate the buoyant density of Ba1 (ca. 1.55 g/ml), and a working gradient was established that consisted of three steps (1.36 g/ml, 1.50 g/ml, and 1.60 g/ml). The three-step gradient produced a well-isolated,

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TABLE 1. Bacterial and bacteriophage strains

Strain	Description	Source or reference
<i>B. avium</i>		
197N	Parental strain; relevant phenotype: Ba1 ^s Ba2 ^s NaI ^r Str ^s Lac ⁻ Kan ^s Mot ⁺ Hag ⁺	37
197N2	Spontaneous streptomycin-resistant mutant of 197N	This study
G145	197N except <i>hag::mini-Tn5lacZ2</i> Hag ⁻ Kan ^r Lac ⁺	This study
G146	197N except <i>mot::mini-Tn5lacZ2</i> Mot ⁻ Kan ^r Lac ⁺	37
1B1	197N with random insertion (Ω 1) using mini-Tn5	This study
1B4	197N with random insertion (Ω 2) using mini-Tn5	This study
1B5	197N with random insertion (Ω 3) using mini-Tn5	This study
AP21	197N lysogenized by Ba1	This study
GOBL271 ^a	Strain screened for temperate phage (Ba1 and Ba2, sensitive)	15
35086	Strain screened for temperate phage (lysogenic for Ba2; resistant to Ba1 and Ba2)	ATCC ^b
Wampler	Strain screened for temperate phage (lysogenic for Ba1; Ba2 sensitive)	22
F247-P4164	Clinical isolate; Ba1 sensitive	J. K. Skeeles
F242-95-950	Clinical isolate; Ba1 insensitive	J. K. Skeeles
F241-95-931	Clinical isolate; Ba1 sensitive	J. K. Skeeles
F243-P4485	Clinical isolate; Ba1 sensitive	J. K. Skeeles
TR96-1212	Clinical isolate; Ba1 insensitive	M. Blakeley
Ba169	Clinical isolate; Ba1 sensitive	19
Ba198	Clinical isolate; Ba1 sensitive	19
Ba177	Clinical isolate; Ba1 insensitive; lysogenic	19
Ba002	Clinical isolate; Ba1 sensitive	19
<i>B. hinzii</i>		
Ba008	Clinical isolate; Ba1 insensitive	Y. M. Saif
<i>B. bronchiseptica</i>		
17640	Clinical isolate; Ba1 insensitive	23
110NH	Clinical isolate; Ba1 insensitive	4
110H	Clinical isolate; Ba1 insensitive	4
R-5	Clinical isolate; Ba1 insensitive	6
Romark	Clinical isolate; Ba1 insensitive	24
87	Clinical isolate; Ba1 insensitive	4
64-C-0406	Clinical isolate; Ba1 insensitive	Laboratory collection
JS34682	Clinical isolate; Ba1 insensitive	24
BB213	Clinical isolate; Ba1 insensitive	E. Hewlett
BB361	Clinical isolate; Ba1 insensitive	24
<i>B. pertussis</i> 388	Ba1 insensitive	40
<i>B. paraptussis</i> 253	Ba1 insensitive	25
Bacteriophage		
Ba1	Wild type; isolated from <i>B. avium</i> strain Wampler	This study
Ba1c1	Clear-plaque mutant of Ba1	This study
Ba2	Temperate <i>B. avium</i> phage isolated from ATCC strain 35086	This study

^a GOBL271 is derived from the same lineage as 197N (i.e., the parental strain of both is 197 [14]). This relationship was unknown at the time of screening for bacteriophage.

^b American Type Culture Collection, Manassas, Va.

readily visible phage band when approximately 10^{11} PFU were applied to the gradient. Needle aspiration of the band resulted in the recovery of an average of $75\% \pm 15\%$ of the PFU applied to the gradient. Phage DNA was extracted as indicated for bacteriophage λ (24). Chromosomal and plasmid DNA was isolated as described for *Escherichia coli* (32). Restriction endonuclease digestion and agarose gel electrophoresis were performed as described by Davis et al. (10). DNA probes were isolated from agarose gels and then labeled with digoxigenin (DIG) as described by the manufacturer (Boehringer Mannheim). Transfer of DNA to nitrocellulose was accomplished as directed by Maniatis et al. (23), and bands were detected as directed in the DIG high-prime DNA labeling and detection starter kit II (Boehringer Mannheim).

Electron microscopy. Phage morphology measurements were conducted using CsCl purified phage. For contour length phage genome measurements, dilutions of phage DNA (extracted as directed by Dykstra [12]) were mixed with dilutions of purified pBR322 DNA (used as a size standard). These mixtures were placed on Formvar-coated grids and treated as described by Dykstra (12). Grids were examined with a Philips 410 transmission electron microscope. Contour lengths were determined for 10 full-length phage genomes using a map measure and were compared to the lengths of relaxed forms of pBR322.

Immunological techniques. Antibody to Ba1 was raised by injection of a New Zealand White rabbit with approximately 10^{11} PFU of CsCl-purified phage in

complete Freund's adjuvant. Six monthly booster doses of approximately 10^{10} PFU in incomplete Freund's adjuvant were given, and blood was withdrawn biweekly. The neutralization constant (*K*) value (42) of the antiserum after 2 months was stable at approximately 470 min^{-1} .

Genetic techniques. Coreversion analysis of a motility-negative (Mot⁻) mini-Tn5lacZ insertion mutant (strain G146 [Table 1 and reference 37]) that was phenotypically Lac⁺ on X-Gal plates was accomplished by first patching 20 colonies into a 0.35% soft agar layer on BHI agar plates. This was followed by examination of the plates after 48 h at 31°C for flares of motile revertants surrounding the original site of inoculation. Twenty independently isolated, motile revertants were examined for their Kan and Lac phenotype. All were Kan^s, Lac⁻ and Mot⁺.

Transduction experiments were carried out with donor strains that had mini-Tn5 insertions (11). Recipient strains were grown overnight in BHI broth, and 0.2 ml of that culture was mixed with 0.1 ml of serially diluted crude phage lysate (average titer 5×10^9 PFU/ml) to produce a range of ratios from approximately 0.1 to 0.001 PFU/CFU in 10-fold increments. After a 20-min adsorption period at 37°C, the mixtures were diluted with 1.0 ml of BHI broth containing 0.1 M sodium citrate, and the bacteria were isolated by a brief centrifugation. The bacterial pellets were resuspended in 1.0 ml of the same medium and were incubated with shaking for 1.0 h at 37°C. After this incubation (to allow pheno-

typic expression of the *neoR* gene in mini-Tn5), cells were reisolated by centrifugation, and each cell pellet was resuspended in 0.15 N NaCl (0.1 ml). Each suspension was plated on BHI agar containing 40 µg of kanamycin/ml.

UV irradiation of phage lysates was accomplished using a General Electric G8T5 UV bulb and exposing approximately 12 ml of freshly prepared crude lysate (diluted to give approximately 2×10^9 PFU/ml in 0.15 N NaCl) in the bottom of a plastic petri plate. Ergs/mm² were calculated using a Blak-Ray model J225 UV light monitor.

Ba1 lysogens of strain 197N were isolated by first spotting a drop of Ba1 phage lysate onto a lawn of strain 197N. After 8 h of incubation at 37°C, material from the turbid area where Ba1 had been spotted was streaked to obtain isolated colonies. Such isolates were defined as lysogens by (i) their resistance to lysis by Ba1 in cross-streaking tests (32) and (ii) their ability to produce phage as detected by the presence of PFU in supernatants from broth cultures. Lysogeny was confirmed physically by DNA hybridization using DNA from CsCl-purified phage as a probe.

Hydroxylamine mutagenesis of Ba1 to produce clear plaque mutants was accomplished essentially as described previously for λ phage (10). Pilot experiments revealed that hydroxylamine exposure at 37°C for 15 h produced the largest proportion of clear plaque mutants.

Spontaneous streptomycin-resistant mutants (Str^r) were isolated by plating approximately 10^{10} CFU of strain 197N on BHI agar medium containing 50 µg of streptomycin/ml and were single-colony purified. Random insertion mutants and the hemagglutination defective (Hag⁻) insertion mutant (strain G145 [Table 1]) were obtained as described by Temple et al. (37). The Hag⁻ mutant contained mini-Tn5*lacZ*2 (11) and was phenotypically Lac⁺ on X-Gal-containing agar. The random insertion mutants contained mini-Tn5Km2 (11).

Statistical analysis. Means, the standard deviation of the means, and the statistical significance of mean differences (using Student's *t* test) were determined with the aid of the statistical package included with Microsoft Excel, version 4.0. *P* values of <0.05 were taken as significantly different.

RESULTS

Discovery. Ba1 was discovered along with another *B. avium* temperate phage (Ba2) in a screen that involved cross-spotting supernatants from four *B. avium* strains (197N, GOBL271, ATCC 35086, and Wampler [Table 1]) onto lawns of each of the strains. Supernatants from broth cultures of the ATCC 35086 strain and the Wampler strain produced plaques on our laboratory *B. avium* strain, 197N. The Wampler phage was designated Ba1, and the ATCC 35086 phage, Ba2. Both phage appeared to be temperate, judging from their turbid plaques. Also, both phage were morphologically indistinguishable when viewed in the electron microscope (data not shown). However, lysogens constructed from each phage in strain 197N were heteroimmune and were distinguishable by other criteria (e.g., plaque morphology [our unpublished observations]). Ba1 was the only phage found to transduce via allelic replacement (described in more detail below) and was consequently chosen for further study.

Host range. A spot test, in which at least 10^8 PFU of Ba1 (in approximately 20 µl) was dispensed onto lawns of *Bordetella bronchiseptica*, *B. pertussis*, *B. hinzi*, and *B. paraptensis*, was used to determine if they were sensitive to Ba1-mediated lysis. None of the species tested (Table 1) showed any evidence of sensitivity to Ba1. However, six of nine *B. avium* strains, obtained from clinical cases of bordetellosis (Table 1), were sensitive to Ba1 in the spot test. (Ba1 was subsequently shown to form plaques on these strains with a plaquing efficiency similar to that seen with strain 197N.) Of the three resistant strains, one (Ba177) appeared to be lysogenic for a bacteriophage morphologically similar to Ba1, raising the possibility that resistance in this strain was due to immunity or to superinfection exclusion (2). However, no experiments were performed to confirm this possibility.

Morphology and genome length of CsCl-purified phage. Measurements of 10 virions negatively stained and examined via transmission electron microscopy produced the following morphological measurements: (i) head size, measured parallel and perpendicular to the tail, was 55 ± 4 nm and 53 ± 2 nm, respectively; (ii) extended tail length was 85 ± 5 nm; and (iii)

extended tail width was 14 ± 2 nm. Electron microscopy further revealed that the phage had a contractile, sheathed tail and faintly visible short tail fibers (Fig. 1A). The length of the double-stranded, linear DNA phage genome was determined by contour length measurements using pBR322 (in its relaxed circular form) as a size standard (Fig. 1B). Measurements of 10 phage genomes produced a length corresponding to a size of 46.5 ± 0 kb. This size is similar to size estimates based on electrophoretic migration of phage DNA digested with three different restriction endonucleases (*Xho*I, *Hind*III and *Eco*RI), which gave an average size of 48.6 ± 2.0 kb (data not shown).

Ba1 prophage state. Ba1 lysogeny was stable in the absence of external reinfection, as demonstrated by the growth of a 197N lysogen for over 25 generations in the presence of Ba1 antiserum sufficient to eliminate all detectable external phage ($K = 47 \text{ min}^{-1}$). Cultures were maintained in logarithmic growth by periodic dilution. Examination of 100 isolated colonies at the beginning and at the end of the experiment for Ba1 production and Ba1 immunity revealed that all 200 colonies examined were lysogenic. From the foregoing, we concluded that Ba1 formed a true lysogen rather than existing as a pseudolysogen (produced as a result of a persistent infection and exemplified by certain filamentous phages [2]).

DNA hybridization studies that compared bacterial DNA from four independently isolated lysogenic 197N strains, the nonlysogenic 197N strain, and vegetative Ba1 DNA strongly suggested that the phage genome was integrated into the chromosome or other stable host replicon (strain 197N has a single large plasmid) at a preferred site on both the host and phage. One such lysogen, identical to the other three examined, is shown in Fig. 2A. From the figure, it is evident that at least one vegetative phage band (the 9.2-kb c band in lane 3) was lost upon lysogeny, and at least one new band appeared in the lysogen (c', lane 2). Whereas this might be consistent with phage circularization, the total molecular weight of the hybridizing bands in the lysogen was higher than that of the total phage DNA, implying some form of integration. When a portion of the vegetative phage c band (an *Eco*RI fragment internal to the *Not*I) was used as a hybridization probe rather than whole vegetative phage DNA, two new bands in the lysogen were clearly present (c' and c'' Fig. 2B). We propose that the vegetative c fragment contains an attachment site which is split in two during integration to produce the c' and c'' fragments in lysogens. The absence of the c'' band in Fig. 2A is likely due to the low specific activity of the whole phage probe DNA. In fact, the c'' band was seen when using the whole phage probe if longer radiographic exposure was employed in concert with shorter electrophoresis times (data not shown). These procedures increased sensitivity and reduced band diffusion, respectively. The nonlysogenic strain 197N contained some DNA similar to Ba1 (lane 1, Fig. 2A and B). This may represent cryptic (nonfunctional) phage DNA or DNA coincidentally similar to the phage. Attempts to induce Ba1 lysogens with UV irradiation or mitomycin C using standard techniques were unsuccessful (our unpublished results).

Ba1-mediated transduction of markers at five randomly chosen loci. In order to test whether Ba1 was a transducing phage, we first established the association of a motility mutation with a transposon insertion using a coreversion test (Materials and Methods). We then used the Mot⁻ Kan^r Lac⁺ mutant as a donor in transduction experiments. The recipient was a spontaneous streptomycin-resistant (Str^r) mutant of strain 197N. Selecting for Kan^r, we readily found Kan^r transductants, all having the phenotype Str^r Kan^r Mot⁻ Lac⁺, as opposed to the donor phenotype, Str^r Kan^r Mot⁻ Lac⁺, or the recipient phenotype, Str^r Kan^s Mot⁺ Lac⁻. Interestingly, the

FIG. 1. (A) Transmission electron micrograph of negatively stained Ba1 phage particles. One phage (arrowhead) appears to have an empty head. (Note its electron-darkened head and contracted tail sheath exposing the inner tail core.) (B) Transmission electron micrograph of Ba1 DNA prepared from purified phage as described in Materials and Methods. Arrowheads denote a single linear genome. The relaxed form of pBR322 DNA (4.36 kb) was used as a size standard (box).

Ba2 phage, when tested in this manner, transduced the antibiotic resistance marker, but approximately half of the transductants were still Mot⁺. As stated earlier, we have not characterized Ba2 further. Subsequent transduction experiments using Ba1, a hemagglutination-defective (Hag⁻) Lac⁺ mutant,

and three additional mutants with insertions at undefined locations supported the generalized nature of the transduction in that all five markers tested were transduced, albeit some at different (i.e., statistically distinguishable) frequencies than others (Table 2).

FIG. 2. (A) An autoradiograph of *NotI*-digested chromosome and phage DNA that was hybridized with DIG-labeled vegetative Ba1 DNA. Lanes: 1, nonlysogenic strain (197N); 2, lysogenic strain (AP21); 3, vegetative Ba1. The numbers to the left denote the fragment sizes in kilobases of the bands lettered on the right. The letters (a to e) identify specific vegetative phage fragments. The c' in the lysogen lane (2) denotes a new fragment in the lysogen not present in the vegetative phage. (B) An autoradiograph of *NotI*-digested chromosomal and phage DNA hybridized with a DIG-labeled *EcoRI* restriction endonuclease fragment internal to the vegetative Ba1 *NotI* c fragment. Lanes: 1, the nonlysogenic strain (197N); 2, lysogenic strain (AP21); 3, vegetative Ba1. The numbers to the left denote the molecular weights of the putative attachment site-containing bands (c in the vegetative phage, and c' and c'' in the lysogen) in kilobases.

Physical evidence for a reciprocal exchange of markers in each of the five transductions came from DNA hybridization experiments using donor, recipient, and transductant DNA digested with *EcoRI* and probed with the Tn903 *neoR* gene (*EcoRI* does not cleave in *neoR* [11]). As was seen in all the transductants, including the two examples shown (*mot*::mini-Tn5 and *hag*::mini-Tn5 [Fig. 3]), donors and transductants had identical banding patterns. This was true also when donor and transductant DNA were digested with restriction endonucleases that cleaved at sites internal to the *neoR* gene (data not shown).

UV irradiation of transducing lysates. One complication of Ba1 transduction was that transductants were frequently lysogenized. Ostensibly, this was because of the relatively high number of phage needed to get a successful transduction. Ba1 antiserum, employed during the transduction, was not effective at improving transduction frequency and eliminating lysogeni-

FIG. 3. Autoradiograph showing *EcoRI* restriction endonuclease fragments of donor and recipient strains in transduction experiments using a *neoR* gene probe as described in Materials and Methods. Lanes: 1, *mot*::mini-Tn5lacZ donor strain (G146); 2, recipient strain (197N2); 3, *mot*::mini-Tn5lacZ transductant; 4, *hag*::mini-Tn5lacZ donor strain (G145); 5, recipient strain (197N2); 6, *hag*::mini-Tn5lacZ transductant. Arrowheads denote the size of the reacting bands.

zation (our unpublished results). However, UV irradiation of Ba1, so as to drastically reduce the number of viable phage, not only reduced lysogen formation but also greatly increased transduction frequency (Fig. 4). The best transduction frequency with the fewest lysogens was found after exposure of Ba1 phage to approximately 4.2×10^4 ergs/mm², resulting in approximately 99.9% killing of vegetative phage. A similar level of UV irradiation of a hydroxylamine-derived clear plaque Ba1 mutant (Ba1c1) was extremely effective at improving transduction frequency, and transductant lysogeny was eliminated (data not shown). However, unirradiated Ba1c1 lysates did not transduce at measurable frequencies.

DISCUSSION

In this report we describe the discovery and characterization of Ba1, a temperate, transducing phage for *B. avium*. Ba1 was isolated along with one other temperate but distinctly different *B. avium* phage, Ba2. Ba1 was chosen for further study because it was capable of transduction via an allelic replacement mechanism. To our knowledge, Ba1 and Ba2 are the first reported phage discovered for this species of *Bordetella*.

Other members of the *Bordetella* genus are known to be infected by temperate phage (20, 21, 29). Both *B. pertussis* and *B. bronchiseptica* have temperate phage associated with them, and one phage for *B. bronchiseptica* has been reported to be a temperate transducing phage (M. Liu and J. F. Miller, Abstr. 97th Gen. Meet. Am. Soc. Microbiol., abstr. B-321, 1997). However, none of the *Bordetella* phage have been used extensively as genetic tools, nor has an examination of the effect of lysogeny or phage resistance upon virulence been investigated.

Ba1 had an icosahedral head, a sheathed, contractile tail,

TABLE 2. Ba1 transduction frequencies of insertion mutations at various loci^a

Donor strain	Locus ^b	Phenotype conferred	Transductants per PFU (avg $\pm$ SD)
G145	<i>hag</i> ::mini-Tn5lacZ	Hag ⁻ Kan ^r Lac ⁺	$1.7 \times 10^{-8} \pm 2.2 \times 10^{-8}$
G146	<i>mot</i> ::mini-Tn5lacZ	Mot ⁻ Kan ^r Lac ⁺	$1.7 \times 10^{-7} \pm 2.9 \times 10^{-8}$
1B1	Ω 1	Kan ^r	$1.1 \times 10^{-8} \pm 1.0 \times 10^{-8}$
1B4	Ω 2	Kan ^r	$9.0 \times 10^{-8} \pm 8.7 \times 10^{-8}$
1B5	Ω 3	Kan ^r	$2.0 \times 10^{-8} \pm 2.1 \times 10^{-8}$

^a Transductions were carried out using dilutions of crude lysates as described in Materials and Methods. Ba1 lysates were not UV irradiated. All transducing lysates had similar titers (average, $4.8 \times 10^9 \pm 3.7 \times 10^9$ PFU/ml). The alleles transduced at the two highest frequencies (*mot* and Ω 2) were statistically distinct from the rest but not from each other.

^b The *hag* and *mot* mutations were conferred by mini-Tn5lacZ transcriptional fusions. All Kan^r transductants (>100 tested) had the hemagglutination-negative (Hag⁻) or motility-negative (Mot⁻) phenotype of the donor (37). Also, the transductant colonies were blue ("Lac⁺") on X-Gal-containing agar, as were the donors. Insertion mutants 1B1, 1B4, and 1B5 have random mini-Tn5 insertions. The insertion sites in these mutants were not mapped except to note that they were each inserted into restriction endonuclease fragments of a different size, as indicated by DNA hybridization studies (data not shown).

FIG. 4. Effect of UV irradiation on transduction parameters: viable phage, transduction frequency, and transductant lysogeny. Solid squares (■) denote the percentage of viable Ba1 after UV irradiation of a crude lysate grown on strain G146. Also plotted are the percentage of *mot::mini-Tn5lacZ* nonlysogenized transductants (●) and the number of transductants emerging from the irradiated lysate at each UV dose (△). Points represent the averages of two experiments. Vertical bars denote standard errors of the means.

double-stranded DNA, and a linear genome of a remarkably constant length. Ba1 most closely resembled members of the *Myoviridae* family morphologically (1). The stability of the prophage state was indicated by using Ba1-specific antiserum to show that lysogeny was maintained in the absence of external reinfection. Results of DNA hybridization experiments with four independently isolated 197N lysogens were consistent with phage circularization and integration at a preferred site in both phage and host replicons (7).

The most useful feature of Ba1 was its ability to transduce genetic markers. This property should allow the transfer of mutations between any Ba1-susceptible *B. avium* strains. Using nine random clinical *B. avium* isolates, we found that 67% were susceptible, but none of the other *Bordetella* species were lysed by Ba1. With regard to transduction frequency, some genetic markers were transduced at frequencies significantly different from those of others. A contributing factor to heterogeneity in marker-based transduction frequencies has been traced to features of the DNA surrounding the marker being transduced (e.g., *chi* sites [27]). Such features may be a contributing factor here. Also, we do not know the distance between the five transduced markers. We have assumed, since all five markers were chosen at random and all five were transduced, that the transduction is generalized. However, should the markers all turn out to be (coincidentally) closely linked, this conclusion would need to be reevaluated.

As with other transducing phage, transduction frequency was dramatically improved by UV irradiation of the transducing lysate (13). UV treatment reduces the number of viable phage, which are responsible for killing or lysogenizing transductants. With regard to the problem of transductant lysogenization, our Ba1 clear-plaque mutant (Ba1c1), if UV irradiated, eliminated the problem of transductant lysogeny. In addition to the ability of Ba1 to transduce, other properties associated with its interaction with *B. avium* (e.g., lysogeny or mutations to Ba1 resistance) may prove to be instrumental in better understanding *B. avium* pathogenesis.

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