Generalized transduction in *Streptomyces coelicolor*

Julie Burke, David Schneider*, and Janet Westpheling†

Genetics Department, University of Georgia, Athens, GA 30602

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We report the isolation of generalized transducing phages for *Streptomyces* species able to transduce chromosomal markers or plasmids between derivatives of *Streptomyces coelicolor*, the principal genetic model system for this important bacterial genus. We describe four apparently distinct phages (DAH2, DAH4, DAH5, and DAH6) that are capable of transducing multiple chromosomal markers at frequencies ranging from 10^{-5} to 10^{-9} per plaque-forming unit. The phages contain DNA ranging in size from 93 to 121 kb and mediate linked transfer of genetic loci at neighboring chromosomal sites sufficiently close to be packaged within the same phage particle. The key to our ability to demonstrate transduction by these phages was the establishment of conditions expected to severely reduce superinfection killing during the selection of transductants. The host range of these phages, as measured by the ability to form plaques, extends to species as distantly related as *Streptomyces avermitilis* and *Streptomyces verticillus*, which are among the most commercially important species of this genus. Transduction of plasmid DNA between *S. coelicolor* and *S. verticillus* was observed at frequencies of $\approx 10^{-4}$ transductants per colony-forming unit.

It would be difficult to overestimate the contribution generalized transduction has made to the study of prokaryote biology since the discovery of phage P22 in *Salmonella typhimurium* and phage P1 in *Escherichia coli* in the early 1950s (1, 2). Generalized transduction remains an important genetic tool for fine-structure mapping, site-directed mutagenesis, and transposon-related genetic manipulation even in highly developed model systems such as *E. coli*, *S. typhimurium*, and *Bacillus subtilis*. In organisms such as *Streptomyces coelicolor* the need for such genetic tools is even greater. *S. coelicolor* is the most genetically well characterized actinomycete, an extremely diverse group of filamentous prokaryotic organisms, which includes the producers of the majority of therapeutically important natural product antibiotics (3). The mycelial growth mode and sporulation cycle of these unusual bacteria also offers one of the most dramatic examples of prokaryotic morphological differentiation. They grow as multicellular, multinucleoid, branching hyphae that penetrate and solubilize organic material in the soil, forming a mycelial mass. In response to extracellular signals (4), they initiate a cycle of differentiation that begins with the production of aerial hyphae that septate into uninucleoid compartments that give rise to spores. *S. coelicolor*, therefore, has attracted wide interest as a model system for understanding how bacteria regulate changes in gene expression during differentiation and coordinate these changes temporally and spatially with complex changes in cellular morphology (5, 6).

Despite their interesting biology and commercial importance, relatively little is known about the gene expression pathways that regulate morphological development or antibiotic biosynthesis. A major limitation in the study of morphogenesis in *Streptomyces* has been the inability to clone genes identified by morphological mutations. For example, despite intensive efforts to clone and study the *bld* loci, sites of mutations that cause pleiotropic defects in morphological development, antibiotic production, and extracellular signaling, only five *bld* genes have been characterized at the molecular level since the first description of these mutations in 1976 (7–11).

The reasons for such slow progress are that the obvious genetic approaches for recovering genes identified by chemically induced mutations have been difficult to implement in *Streptomyces.* Relatively few genetic markers exist, making fine structure mapping impossible. Cloning by complementation is slow and tedious. Transformation of plasmid libraries constructed in either *E. coli* or *Streptomyces* is inefficient, and the libraries are often incomplete. Transposons indigenous to *Streptomyces* (12, 13) or derived from other bacteria (14, 15) have been identified, but they have not proven effective for insertional mutagenesis in *S. coelicolor*, in part due to the nature of transposon delivery systems currently available that typically depend on temperature-sensitive plasmid vectors. Curing is not effective, and exposure to high temperatures is mutagenic, which results in a high background of mutations not caused by transposition. Mutations resulting from transposition are not easily distinguished from this background because the genetic tools have not been available to establish causal relationships between transposon insertions and mutant phenotypes. Gehring *et al.* (16) recently have developed a method for efficient *in vitro* transposition of Tn5 in *S. coelicolor* that takes advantage of chromosomal transformation (17) to show linkage between phenotypes of interest and transposon insertions. Cotransformation, however, may not be efficient enough to allow genome scale screening of transposon-generated mutations.

It was widely recognized that an efficient system for generalized transduction was needed in *Streptomyces*, but attempts to identify such phages for *S. coelicolor* were unsuccessful, as were attempts to transduce markers by the most extensively studied *Streptomyces* phages PhiC31, VP5, and R4 (18). A generalized transducing phage was reported for *Streptomyces venezuelae* (19), but it was thought to be an anomaly and somehow specific to *S. venezuelae* because the approaches used to identify transducing phages for *S. venezuelae* did not work for *S. coelicolor*. It was even suggested that most streptomycetes lacked a host factor necessary for the propagation of generalized transducing phages and were thus incapable of supporting generalized transduction (20).

Here we report the isolation and partial characterization of four phages that are capable of efficient generalized transduction in *S. coelicolor*. These phages exhibit a broad host range and will very likely be useful for genetic manipulation of other *Streptomyces* spp. At least three of the phages transduce plasmids between strains of *S. coelicolor* and other *Streptomyces* spp. The ability to transduce plasmids from *S. coelicolor* to *S. verticillus*, for example, a species that has been refractory to genetic manipulation will allow genetic analysis of the bleomycin biosynthetic pathway. We also describe methods for minimizing superinfection killing during the selection of transductants that may allow the isolation and characterization of generalized transducing phages for any *Streptomyces* spp.

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Abbreviations: APB, actinomycete phage buffer; cfu, colony-forming unit; pfu, plaque-forming units.

*Present address: Department of Bacteriology, University of Wisconsin, Madison, WI 53706.

†To whom reprint requests should be addressed. E-mail: janwest@arches.uga.edu.

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Table 1. *S. coelicolor* strains in this study

A3(2)*	Wild type
J2402*	*whiB*::*hyg*
1258*	*proA1 argA1 cysD18 hisC9 uraA1 strA1 NF*
J2408*	*whiH*::*ermE*
YU105†	*proA1 argA1 redE60 act*::*ermE whiE*::*hyg*
BldK::Ω†	*bldK*::*spc*/*str*
J222*	*uraA1 rifA1* NF
2709*	*proA1 argA1 cysD18 hisA1 uraA1 strA1*

*Obtained from Keith Chater, John Innes Centre, Norwich, UK.
†Obtained from Justin Nodwell, McMaster University, Hamilton, Ontario.

Table 2. Phage properties

Phage	Plaque size	DNA MW	Enzyme/#fragments/MW	
DAH1	<0.5 mm	117 kb	*Eco*RV (25)	117
			*Nco*1 (22)	117
DAH2	0.5 mm	121 kb	*Eco*RV (23)	122
			*Cla*1 (13)	121
DAH4	2.0 mm	118 kb	*Nco*1 (20)	115
			*Eco*RV (22)	121
DAH5	1.5 mm	120 kb	*Xba*1 (14)	122
			*Eco*R1 (18)	118
DAH6	1.0 mm	93 kb	*Xba*1 (13)	93
			*Eco*RV (19)	93

Plaque sizes measured on a lawn of *S. coelicolor* A3(2) at 28°C.

Experimental Procedures

Bacterial Strains and Culture Conditions. *S. coelicolor* strains used in this study are listed in Table 1. Standard techniques were used for growth and manipulation (21). Spore stocks were made from strains grown on maltose-yeast extract-malt extract agar (22).

Isolation of Phages. Samples of topsoil (25 g) were incubated with 15 ml of actinomycete phage buffer (APB) (23) overnight at room temperature on a rocking shaker. The mixture was centrifuged at 1,100 × *g* for 10 min, and the supernatant was passed through a 0.2-μm Nalgene cellulose acetate filter and stored at 4°C. To detect phage, 100 μl of filtrate was added to *S. coelicolor* spores diluted to $\approx 10^7$ colony-forming unit (cfu)/ml, in 4 ml of nutrient soy top agar (23) and poured over nutrient agar plates containing 4 mM $Ca(NO_3)_2$ and 0.5% dextrose (19). Plates were incubated at 30°C for 3 days and examined for plaques. Phages were isolated by three rounds of plaque purification.

Preparation of Phage Stocks. Phages were propagated on donor strains by standard agar-layer techniques (24) with APB used as phage diluent. To prepare lysates $\approx 10^4$ phage were added to $\approx 10^6$ spores in 4 ml of nutrient soy top agar, poured onto nutrient agar plates containing 4 mM $Ca(NO_3)_2$ and 0.5% dextrose, and incubated for 2–5 days at 25°C. Phages were collected from the top agar, and bacterial contamination was eliminated by filtration through a 0.2-μm cellulose acetate filter. Phage titers [the number of plaque-forming units (pfu) per ml of lysate] were determined by spotting 20 μl of phage diluted in APB onto lawns of spores on nutrient soy top agar. Titer plates were incubated overnight at 30°C. High titer lysates are essential. Typically lysates of at least 10^{10} pfu/ml are used (at an multiplicity of infection of 10) for transduction assays.

Inactivation of Phage with Citrate. To determine the ability of the phage to form plaques on sodium citrate, the titer of each phage was determined by spotting 20 μl of phage diluted in APB onto a lawn of *S. coelicolor* spores plated on nutrient soy top agar with and without 10 mM sodium citrate. Plates were incubated at 25°C or 30°C for 1 or 3 days and examined for plaques.

Preparation of Germinated Spores. Spores were incubated first at 50°C for 10 min in 0.05 M TES buffer (21), then at 30°C for 2 h with addition of an equal volume of 2× germination broth (21). The emergence of germ tubes from spores was monitored by examination in a light microscope. Germlings were collected by centrifugation for 5 min at 2,000 × *g*, resuspended in water, and plated immediately.

Genetic Transduction Assays. High titer ($>10^{10}$ pfu/ml) phage lysates were prepared on donor strains as described above, diluted in APB, added to pregerminated recipient spores at a multiplicity of infection of 1–100 (typically 10), and incubated for 30 min at room temperature, then spread on supplemented minimal medium (21) or nutrient agar plates containing 4 mM $Ca(NO_3)_2$ and 0.5% dextrose containing antibiotic and incubated 5–7 days at 22°C. Transduction frequencies were calculated as the number of colonies per pfu (cfu/pfu) as well as the number of colonies per cfu (cfu/cfu) in the infection mixture. To reduce superinfection, the phage-germlings mixture was plated on minimal medium that contained 10 mM sodium citrate and incubated at 22°C instead of 30°C, the optimal growth temperature for these strains. Please note that the level of drug required for selection of germlings is significantly different from that for spores and the levels required vary, depending on the strain. To test for reversion, germlings suspended in APB were incubated exactly as those with phage and plated on selective media.

Phage DNA Isolation and Characterization. Phage were prepared essentially as described for phage lambda (25). Phage particles were precipitated from lysates for 16 h at 4°C by using polyethylene glycol (PEG 8000) at a final concentration of 10%, pelleted by centrifugation (4,000 × *g*, 10 min, 4°C), and resuspended in suspension medium buffer with KCl added to a final concentration of 1 M. After centrifugation (12,000 × *g*, 10 min, 4°C) to remove the polyethylene glycol, the phage-containing supernatant was layered onto a preformed CsCl gradient (3.5 ml 1.7 g/ml, 2.5 ml 1.5 g/ml, 2.5 ml 1.3 g/ml) and centrifuged (50,000 × *g*, 2 h, 4°C) to concentrate phage particles. Phage were collected from the gradient and filtered (Microcon-100 with a molecular weight cutoff of 100,000; Amicon) to remove CsCl. DNA was extracted with phenol and precipitated by using 70% ethanol, 10 mM Tris·HCl (pH 7.6), and 1 mM EDTA.

Results and Discussion

Isolation of Phages. *S. coelicolor* is a common saprophytic soil bacterium. To recover phage isolates that might form plaques on a lawn of *S. coelicolor*, 26 soil samples from different locations and soil environments in the vicinity of Athens, GA were extracted with phage buffer, passed through a 0.2-μm cellulose acetate filter, and tested for the presence of pfu by plating in soft agar overlays seeded with $\approx 10^7$ *S. coelicolor* spores. Approximately one-third of these samples (8 samples) yielded at least one plaque. Unexpectedly, clay loam samples were a much better source of phages (eight of eight samples tested) than rich humus (zero of eight samples tested). Single-plaque suspensions were prepared by three rounds of plaque purification from one or two plaques per soil sample and used to generate plate lysates by harvesting the top agar overlay from plates of suspension dilutions that produced nearly confluent lysis. In total, six lysates were prepared, yielding initial titers ranging from 10^3 to 10^5 pfu/ml and in all cases producing relatively large plaques at 30°C. At ambient laboratory temperature (≈22°C), however, several of the lysates produced very turbid plaques with a range of plaque morphologies. Six such turbid plaque-forming isolates, DAH1–6 (Table 2), differing somewhat from each other in plaque mor-

Table 3. Sensitivity of plaque formation to temperature and sodium citrate

Phage	− Citrate 22° C	+ Citrate 22° C	− Citrate 30° C	+ Citrate 30° C
After 1 days growth				
DAH1	NPD	NPD	2.5×10^7 clear plaques	500 turbid plaques
DAH2	NPD	NPD	2.5×10^7 clear plaques	4×10^5 turbid plaques
DAH4	NPD	NPD	5×10^9 clear plaques	5×10^6 turbid plaques
DAH5	NPD	NPD	1.5×10^9 clear plaques	5×10^7 turbid plaques
DAH6	NPD	NPD	1×10^6 clear plaques	5×10^3 turbid plaques
After 3 days growth				
DAH1	1×10^5 turbid plaques	NPD	2.5×10^7 clear plaques	500 turbid plaques
DAH2	3×10^6 turbid plaques	10 turbid plaques	2.5×10^7 clear plaques	4×10^5 turbid plaques
DAH4	2.5×10^7 turbid plaques	400 turbid plaques	5×10^9 clear plaques	5×10^6 turbid plaques
DAH5	5×10^6 turbid plaques	300 turbid plaques	1.5×10^9 clear plaques	5×10^7 turbid plaques
DAH6	1×10^5 turbid plaques	75 turbid plaques	1×10^6 clear plaques	5×10^3 turbid plaques

NPD, no plaques detected.

phology at 22°C, were selected for further physical and genetic analysis.

Physical Characterization of Phages. To estimate the genome sizes of DAH1–6 and to evaluate their relatedness to each other, DNA samples isolated from each phage were digested with 22 different restriction enzymes and fractionated by agarose gel electrophoresis (data not shown). Initial results indicated that DAH3 and DAH5 were the same phage and that DAH1, DAH2, DAH4, DAH5, and DAH6 were different from each other (Table 2).

Tests for Generalized Transduction. To determine whether any of the phages might be capable of generalized transduction, DAH1, DAH2, DAH4, DAH5, and DAH6 lysates were tested for the ability to transfer genetic markers. In the initial test, donor lysates prepared on strain J222 (*uraA1 rifA1*) were used to infect recipient strain 2709 (*proA1 argA1 cysD18 hisA1 uraA1 strA1*). The recipient was chosen, in part, because reversion of its auxotrophic markers occurs at very low frequency (21). Mutation to rifampicin resistance (conferred by *rifA1*) also occurs at relatively low frequency (26). This combination of strains thus provided the opportunity to test with a high degree of sensitivity for transfer of genetic markers at four widely scattered map locations.

When initial attempts to detect transduction were unsuccessful, we considered the possibility that superinfection killing might be responsible. Efficient adsorption of many phages requires the presence of divalent cations, and phage growth is often reduced at low temperature. Thus superinfection killing is often reduced by plating infection mixtures in the presence of a chelating agent such as citrate or EGTA at low temperature (27, 28). As shown in Table 3, for all five phages the ability to form plaques was very sensitive to citrate, and the inhibitory effects of citrate were much more pronounced at 22°C. The additive inhibitory effects of citrate and low temperature were reflected in plaque size, turbidity, and rate of plaque development.

When transduction tests were repeated in the presence of 10 mM sodium citrate, at 22°C, an indication of apparent transduction above the background of marker reversion was obtained for four of the phages. The highest levels of apparent transduction were observed with DAH4, DAH5, and DAH6, which yielded transductants at frequencies ranging from 10^{-5} to 10^{-7} cfu/pfu for eight selected markers (Table 4). No transduction was observed for DAH1. Reversion to prototrophy of the *proA1, argA1*, or *hisA1* alleles was not detected (less than 10^{-8} cfu/cfu), and spontaneous resistance to rifampicin was observed at a frequency of 1×10^{-8} cfu/cfu, whereas presumptive transductants arose at frequencies of more than 10^{-6} cfu/cfu. The transduction frequency was, therefore, in some cases more than 300-fold greater than the background of mutation to resistance/reversion to prototrophy. These data clearly indicate that colonies arose by phage-mediated DNA transfer.

The genetic markers in J222 and 2709 had been positioned on the *S. coelicolor* genetic map by earlier conjugation-mediated mapping studies (3) and were expected to be unlinked by transduction with DAH4. This expectation was confirmed by testing all presumptive transductants from each cross for the presence of the unselected markers of the recipient. In all cases, transductants retained all recipient parental genotypes except those associated with the selected marker contributed by the donor. No transduction was detected above the background of marker reversion when selections were carried out at 30°C or in the absence of citrate. Detection of transductants with *S. coelicolor* also required the use of recipient spore germlings in the infection mixture. Spore germlings were produced by standard methods involving a 10-min incubation at 50°C followed by 2 h at 30°C (21). Attempts to detect transduction with recipient mycelial fragments or spores, instead of germlings, were unsuccessful (data not shown).

Cotransduction of Genetic Markers. Transfer of markers from multiple chromosomal locations at similar frequencies implies that genetic exchange is mediated by generalized rather than specialized transduction. Moreover, apparent levels of transduction mediated by these phage were at least 10-fold above the background of marker reversion and frequently more than 100-fold above background. Nevertheless, rigorous proof that marker transfer is occurring by the mechanism of generalized transduction requires the demonstration that markers close enough to be packaged within the same phage particle show some degree of cotransfer. To test whether DAH4, DAH5, and DAH6 could mediate linked transfer of markers, we tested genetic linkage of a streptomycin resistance allele (*strA1*) and a rifampicin resistance allele (*rifA1*) that were very likely to be within cotransduction distance of each other. In previous low-resolution conjugation mapping studies, *strA1* and *rifA1* were not separable by recombination (26). More recent physical-genetic mapping studies have placed these markers closer than 7 kb apart (www.sanger.ac.uk/Projects/S_coelicolor), well within the estimated packaging capacity of these phages. To test for cotransduction of these markers, phage lysates were prepared on donor strain 1258 (*strA1*) and used to infect recipient strain J222 (*rifA1*) with selection for streptomycin resistance. As shown in Table 5, all three phages mediated cotransduction of markers at similar

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Table 4. Generalized transduction by DAH2, DAH4, DAH5, and DAH6

Donor	Recipient	Selection	Reversion frequency of selected marker	#Colonies	Frequency, cfu/cfu	Frequency, cfu/pfu
DAH2						
J222	2709	Arg+	$<1 \times 10^{-7}$	11	1×10^{-6}	3×10^{-7}
J222	2709	Pro+	$<1 \times 10^{-7}$	10	1×10^{-6}	3×10^{-6}
J222	2709	His+	$<1 \times 10^{-7}$	31	3×10^{-6}	8×10^{-7}
J222	A3(2)	Rif^r	1×10^{-8}	34	3×10^{-7}	5×10^{-7}
1258	J222	Str^r	3×10^{-8}	0	0	0
YU105	BldK::Ω	Linc^r	1×10^{-8}	0	0	0
YU105	J222	Hyg^r	1×10^{-6}	401	4×10^{-5}	3×10^{-5}
YU105	J2402	Ery^r	1×10^{-7}	62	6×10^{-6}	7×10^{-9}
DAH4						
J222	2709	Arg+	$<1 \times 10^{-8}$	310	3×10^{-6}	5×10^{-6}
J222	2709	His+	$<1 \times 10^{-8}$	210	2×10^{-6}	5×10^{-5}
J222	2709	Pro+	$<1 \times 10^{-8}$	120	1×10^{-6}	3×10^{-6}
J222	A3(2)	Rif^r	1×10^{-8}	123	1×10^{-6}	3×10^{-6}
1258	J222	Str^r	3×10^{-8}	409	4×10^{-6}	2×10^{-6}
YU105	J2402	Ery^r	1×10^{-8}	311	3×10^{-6}	2×10^{-7}
DAH5						
J222	2709	Arg+	$<1 \times 10^{-8}$	12	1×10^{-7}	3×10^{-6}
J222	2709	His+	$<1 \times 10^{-8}$	10	1×10^{-7}	3×10^{-7}
J222	2709	Pro+	$<1 \times 10^{-8}$	21	2×10^{-7}	7×10^{-6}
J222	A3(2)	Rif^r	1×10^{-8}	30	3×10^{-7}	7×10^{-6}
1258	J222	Str^r	3×10^{-8}	31	3×10^{-6}	2×10^{-6}
YU105	BldK::Ω	Linc^r	1×10^{-8}	30	3×10^{-7}	2×10^{-8}
YU105	J222	Hyg^r	1×10^{-6}	62	6×10^{-6}	5×10^{-6}
YU105	J2402	Ery^r	1×10^{-8}	93	9×10^{-7}	2×10^{-7}
DAH6						
J222	2709	Arg+	$<1 \times 10^{-8}$	21	2×10^{-7}	5×10^{-6}
J222	2709	His+	$<1 \times 10^{-8}$	30	3×10^{-7}	7×10^{-6}
J222	2709	Pro+	$<1 \times 10^{-8}$	23	2×10^{-7}	5×10^{-6}
J222	A3(2)	Rif^r	1×10^{-8}	123	1×10^{-6}	3×10^{-6}
1258	J222	Str^r	3×10^{-8}	33	3×10^{-7}	7×10^{-6}
YU105	BldK::Ω	Linc^r	1×10^{-8}	30	3×10^{-7}	3×10^{-8}
YU105	J222	Hyg^r	1×10^{-6}	403	4×10^{-5}	7×10^{-5}
YU105	J2402	Ery^r	1×10^{-8}	42	4×10^{-7}	3×10^{-9}

Rifampicin 150 μg/ml; streptomycin 25 μg/ml; erythromycin 100 μg/ml; hygromycin 25 μg/ml; lincomycin 150 μg/ml.

frequencies. For DAH4, among 335 transductants tested, 225 proved to be rifampicin sensitive, indicating 67% cotransduction of these markers. The cotransduction frequency with DAH6 was 63% (232 of 370). The cotransduction frequencies were the same regardless of the selected marker. The relatively high linkage observed in these experiments agrees with the known physical distance between the *rpsL* (*strA1*) and *rpoB* (*rifA1*) genes.

To further evaluate cotransduction by these phages, we tested for genetic linkage of two constructed alleles, *bldK*::*spc* (a spectinomycin resistance gene integrated into the *bldK* locus) and *act*::*ermE* (an erythromycin resistance gene integrated into the *act* locus) (29). These two alleles are known by DNA sequence analysis to be 25 kb apart in the *S. coelicolor* chromosome (L. Nodwell and R. Losick, personal communication). To test for linkage, phage lysates were prepared on donor strain YU105 (*act*::*ermE whiE*::*hyg*) and used to infect recipient strain BldK::Ω (*bldK*::*spc*), with selection for lincomycin resistance. For DAH4 of 163 transductants tested, 87 became spectinomycin

Table 5. Cotransduction by DAH4, DAH5, and DAH6

Phage	Donor	Recipient	Selection	Spontaneous resistance to selected marker	#Colonies	Transduction frequency, cfu/cfu	Unselected marker	#Colonies	Cotransduction frequency, %
DAH4*	1258	J222	Str^r	$<1 \times 10^{-8}$	335	7.4×10^{-7}	Rif^s	225	67
DAH4*	J222	1258	Rif^r	2.9×10^{-8}	67	9.3×10^{-7}	Str^s	39	59
DAH6*	1258	J222	Str^r	$<1 \times 10^{-8}$	370	9.9×10^{-6}	Rif^s	232	63
DAH6*	J222	1258	Rif^r	2.9×10^{-8}	78	6.7×10^{-7}	Str^s	51	66
DAH4†	YU105	BldK::Ω	Linc^r	1.1×10^{-8}	163	3×10^{-7}	Spc^s	87	53
DAH5†	YU105	BldK::Ω	Linc^r	1.1×10^{-8}	213	3×10^{-7}	Spc^s	125	59
DAH6†	YU105	BldK::Ω	Linc^r	1.1×10^{-8}	142	3×10^{-8}	Spc^s	56	39

*Streptomycin selected at 25 μg/ml; rifampicin test for sensitivity at 50 μg/ml; rifampicin selected at 40 μg/ml; streptomycin test for sensitivity at 40 μg/ml.
†Lincomycin selection at 150 μg/ml; test for spectinomycin sensitivity at 50 μg/ml.

Table 6. Phage host range-plating efficiency of DAH phages on other actinomycetes

Phage	Titer	*S. coelicolor* A3(2)	*S. lividans* 66	*S. avermitilis* 31272	*S. verticillus* 15003	*S. venezuelae* 10712
DAH2	7×10^{9}	1	1	8.5×10^{-2}	NPD	6.8×10^{-7}
DAH4	1×10^{11}	1	3.0×10^{-1}	3.0×10^{-2}	NPD	NPD
DAH5	2×10^{11}	1	3.0×10^{-2}	5.0×10^{-3}	2.5×10^{-8}	1.0×10^{-7}
DAH6	1×10^{11}	1	2.0×10^{-1}	5.0×10^{-2}	6.7×10^{-8}	2.0×10^{-7}

Phage lysates were prepared on *S. coelicolor* A3(2); plating efficiency is reported as the number of pfu observed on the host indicated, divided by the number of pfu in the lysate prepared on *S. coelicolor*. NPD, no plaques detected.

resistant (53% cotransduction). DAH5 indicated 59% (125 of 213) and DAH6 indicated 39% (16 of 42) cotransduction of these markers. Transductants also were tested for transfer of the hygromycin resistance gene present in YU105, which was not expected to be cotransduced. None of the transductants acquired the *whiE*::*hyg* marker.

Host Range of Generalized Transducing Phages. To determine whether DAH2, DAH4, DAH5, or DAH6 might plaque on other *Streptomyces* spp. and, therefore, might be useful for generalized transduction in those species, high-titer phage suspensions were spotted onto soft agar overlays seeded with spores of *S. lividans*, *S. avermitilis, S verticillus,* or *S. venezuelae*. As shown in Table 6, all four lysates gave plaques on *S. lividans* with moderately reduced efficiency relative to *S. coelicolor*. Somewhat surprisingly, all four lysates also gave plaques on *S. avermitilis*, the avermectin producer, with only moderately reduced efficiency. The efficiency of plating of these phage on both *S. venezuelae* and *S. verticillus* was dramatically reduced.

Plasmid Transduction. To evaluate the potential for DAH2, DAH4, DAH5, and DAH6 to mediate transfer of plasmids among *Streptomyces* strains, lysates were prepared on either *S. coelicolor* or *S. lividans* containing either pXE60 (31), a low copy number plasmid encoding thiostrepton resistance (17.5 kb) or pIJ702 (32), a high copy number plasmid (5.8 kb) and used to infect a variety of plasmid-free strains. Repeated attempts to transduce pIJ702 were unsuccessful; however, selection of thiostrepton resistant colonies resulted in the detection of putative transductants on several strains suggesting transfer of pXE60, which contains a thiostrepton resistance marker (31). As shown in Table 7, transduction of pXE60 between strains of *S. coelicolor* was at least an order of magnitude above the frequency of spontaneous resistance. Of particular interest was the ability to transduce plasmid DNA into *S. verticillus*, the bleomycin producer, which has been completely refractory to genetic analysis. As shown in Table 7, DAH5 was efficient in transducing pXE60 from *S. lividans* to *S. verticillus* (2 orders of magnitude greater than the frequency of spontaneous resistance to thiostrepton). Plasmid DNA was not recovered from this strain but because there are no reports of plasmids having been introduced into or isolated from *S. verticillus,* we assume that failure to isolate plasmid DNA is likely to be a technical problem. Southern hybridization analysis using pXE60 DNA as probe, however, clearly showed that pXE60 was present in transductants of *S. verticillus* but not in the wild-type strain (data not shown).

Discussion

From a relatively small number of soil samples from the vicinity of Athens, GA, we isolated five different bacteriophages and demonstrated that all five of them are capable of mediating generalized transduction in *S. coelicolor* at frequencies on the order of 10^{-6} per cfu, roughly equivalent to that of wild-type phage P22 in *Salmonella* (33). We speculate that our success reflects the use of multiple measures for minimizing "superinfection killing" by lethal reinfection of potential transductants by viable phage released into the environment in which transductants are selected. For example, we were only able to detect transduction when transduction mixtures were plated at 22°C (8° lower than the optimal growth temperature of this strain).

Of particular importance from the standpoint of the potential for DAH2, DAH4, DAH5, and DAH6 (or related phages) to be useful in the broader field of actinomycete biology is the fact that these phages appear to have a broad host range. The breadth of this host range was not evident until very high-titer lysates were used to detect plaques on lawns of *Streptomyces* spp. distantly related to *S. coelicolor,* such as *S. avermitilis* or *S. venezuelae*. This low frequency of plaque formation across phylogenetic distance is very likely to be explained by the well-documented prevalence of restriction-modification systems among the actinomycetes (31), which might easily obscure the true replication host range of either phages or plasmids. Indeed, the *S. venezuelae* phage SV1 was previously believed to have a very limited host range (19); yet we have found that high-titer lysates of SV1 do produce rare plaques on *S. coelicolor* (J.B., unpublished work) probably reflecting low-frequency escape from host restriction. It also should be noted that our efforts to detect generalized transducing phages were not extensive as only a small number of soil samples were examined. Thus, it may be that the discovery of other transducing phages even more useful for the many and diverse actinomycetes species of industrial interest might be achieved if careful attention is given to the phenomenon of superinfection killing. The ability to transduce plasmids or even chromosomal markers from *S. coelicolor* to genetically uncharacterized strains of the genus will facilitate, and in some cases, enable genetic manipulation of pathways that lead to the production of therapeutically important antibiotics. For example, *S. verticillus*, which produces bleomycin, an important anticancer chemotherapeutic drug, has proved completely refractory to genetic analysis. There are no existing methods for mating, DNA transformation, or mutational analysis in *S. verticillus*, and the ability to introduce constructed deletions and alterations of the bleomycin biosynthetic pathway by transduction will enable the investigation of the unusual chemistry and biology of this interesting and important pathway.

Table 7. Transduction of plasmid pXE60

Phage	Donor	Recipient	Selection	Spontaneous resistance	#Colonies	Frequency cfu/cfu
DAH4	*S. coelicolor* A3(2)/pXE60	*S. coelicolor* BldK::Ω Spc^{r}	Thio^{r}	$<1 \times 10^{-8}$	15	1.5×10^{-7}
DAH5	*S. lividans* 66/pXE60	*S. verticillus* 15003	Thio^{r}	6×10^{-6}	192	2.4×10^{-4}

Thiostrepton selected at 40 μg/ml. For *s. verticillus*; 15 μg/ml for *s. coelicolor*.

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1. Zinder, N. D. & Lederberg, J. (1952) *J. Bacteriol.* **64,** 679–699.
2. Lennox, E. S. (1955) *Virology* **1,** 190–206.
3. Hopwood, D. A. (1999) *Microbiology* **145,** 2183–2202.
4. Willey, J., Santamaria, R., Guijarro, J., Geislich, M. & Losick, R. (1991) *Cell* **65,** 641–650.
5. Schauer, A., Ranes, M., Santamaria, R., Guijarro, J., Lawlor, E., Mendez, C., Chater, K. & Losick, R. (1988) *Science* **240,** 768–772.
6. Chater, K. F. (1993) *Annu. Rev. Microbiol.* **47,** 685–713.
7. Merrick, M. J. (1976) *J. Gen. Microbiol.* **96,** 299–315.
8. Nodwell, J. R., McGovern, K. & Losick, R. (1996) *Mol. Microbiol.* **22,** 881–893.
9. Elliot, M., Damji, F., Passantino, R., Chater, K. & Leskiw, B. (1998) *J. Bacteriol.* **180,** 1549–1555.
10. Pope, M. K., Green, B. & Westpheling, J. (1998) *J. Bacteriol.* **180,** 1556–1562.
11. Lawlor, E. J., Baylis, H. A. & Chater, K. F. (1987) *Genes Dev.* **1,** 1305–1310.
12. Baltz, R. H., Hahn, D. R., McHenney, M. A. & Solenberg, P. J. (1992) *Gene* **115,** 61–65.
13. Olson, E. R. & Chung, S. T. (1988) *J. Bacteriol.* **170,** 1955–1957.
14. Volff, J. N. & Altenbuehner, J. (1997) *Gene* **194,** 81–86.
15. Herron, P. R., Evans, M. C. & Dyson, P. J. (1999) *FEMS Microbiol. Lett.* **171,** 215–221.
16. Gehring, A. M., Nodwell, J. R., Beverley, S. M. & Losick, R. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 9642–9647. (First Published print August 8, 2000, 10.1073/pnas.170059797)
17. Oh, S. H. & Chater, K. F. (1997) *J. Bacteriol.* **179,** 122–127.
18. Lomovskaya, N. D., Chater, K. F. & Mkrtumian, N. M. (1980) *Microbiol. Rev.* **44,** 206–229.
19. Stuttard, C. (1979) *J. Gen. Microbiol.* **110,** 479–482.
20. Suss, F. & Klaus, S. (1981) *Mol. Gen. Genet.* **181,** 552–555.
21. Kieser, T., Bibb, M. J., Buttner, M., Chater, K. & Hopwood, D. (2000) *Practical Streptomyces Genetics* (The John Innes Foundation, Norwich, UK).
22. Brawner, M. E., Auerbach, J. I., Fornwald, J. A., Rosenberg, M. & Taylor, D. P. (1985) *Gene* **40,** 191–201.
23. Vats, S., Stuttard, C. & Vining, L. C. (1987) *J. Bacteriol.* **169,** 3809–3813.
24. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
25. Yamamoto, K. R., Alberts, B. M., Benzinger, R., Lawhorne, L. & Treiber, G. (1970) *Virology* **40,** 734–744.
26. Chater, K. (1974) *J. Gen. Microbiol.* **80,** 277–290.
27. Ely, B., Weppelman, R. M., Massey, H. C. & Hartman, P. E. (1974) *Genetics* **76,** 625–631.
28. Hodgson, D. A. (1999) *Mol. Microbiol.* **34,** 1–12.
29. Yu, T. W. & Hopwood, D. (1995) *Microbiology* **141,** 2779–2791.
30. Radicio, M. R. & Chater, K. F. (1988) *Gene* **74,** 39–42.
31. Delic, I., Robbins, P. & Westpheling, J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 1885–1889.
32. Katz, E., Thompson, C. J. & Hopwood, D. A. (1983) *J. Gen. Microbiol.* **129,** 2703–2714.
33. Margolin, P. (1987) in *Cellular and Molecular Biology*, ed. Neidhardt, F. C. (Am. Soc. Microbiol., Washington, DC), pp. 1154–1168.