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Inheritance of Nocardiophage φEC in Matings of Nocardial Lysogens

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Lysogens of *Nocardia erythropolis* were mated with nonlysogenic strains to study the inheritance of the φEC prophage. Crosses between lysogenic strains of the Mat-Ce mating type and nonlysogenic Mat-cE strains produced Mat-cE lysogens at a recovery rate of 17%, whereas recombination frequencies between chromosomal traits were about 2.3×10^{-5}. Crosses of lysogenic Mat-cE mating types with nonlysogenic Mat-Ce produced Mat-Ce lysogens at a recovery rate of 19%, whereas recombinants for chromosomal traits were recovered at only 1.8×10^{-5}. Crosses of homologous mating types, lysogenic Mat-Ce with nonlysogenic Mat-Ce or lysogenic Mat-cE with nonlysogenic Mat-cE, failed to transfer the prophage. It was concluded that the φEC prophage exists as a plasmid and can be transferred at high frequencies with patterns of transfer controlled like typical nocardial fertility. Evidence that the prophage may also exist as an integrated element was observed from recombination analyses.

Nocardiophage φEC was isolated from soil samples (6) and used to produce φEC-bearing lysogens of *Nocardia erythropolis* (7). In a preliminary study, matings between lysogenic and nonlysogenic strains indicated that the φEC prophage might exist as a plasmid, since the nonlysogenic parental strains acquired the phage-bearing trait at relatively high frequencies (Proc. 13th Int. Cong. Genet., 1973, Genetics 74: 32s). However, an examination of the recombinant phenotypes produced from these matings revealed that only specific class types had inherited the prophage, suggesting that it exists as an integrated element (G. H. Brownell, Abstr. Annu. Meet. Am. Soc. Microbiol. 1972, G260, p. 74). Although lysogenic strains of *Nocardia* are known to occur (8, 9), plasmids have not been previously reported in this genus. This communication describes the plasmid-like inheritance of the φEC prophage and shows that its inheritance is controlled like nocardial fertility. Strains of homologous origin failed to transfer the plasmid at appreciable frequencies, whereas matings of normally compatible strains permitted transfer of the plasmid at high frequencies.

MATERIALS AND METHODS

Strains. Three independently isolated lysogenic strains of *N. erythropolis* cE2-13 were obtained following infection with phage φEC (7). The lysogenic isolates were designated cE2-13 (φEC)-3, -6, and -8. The strains belong to the Mat-cE mating type (5) and, in addition to bearing the φEC prophage, possess genetic lesions for purine (*purA1*) and histidine (*his-3*) synthesis. Additional lysogens were obtained from mutant substrains of the Mat-Ce mating type. Lysogenic strain Ce3-53 (φEC) requires purines (*purB2*) and arginine (*arg-3*) for growth and is resistant to streptomycin (*strB2*). Strain Ce3-69 (φEC) requires purines (*purB2*) and phenylalanine (*pheA*) for growth.

Mating procedures. Initial crosses were conducted in a U-tube apparatus (parabiotic chambers, Bellco Glass Inc., Vineland, N.J.) to determine if direct cell contact was required for inheritance of the phage-bearing trait. Two chambers, each containing a single mating type, were separated by 0.45-μm membrane filters (Millipore Corp., Bedford, Mass.). Peptone-yeast extract (PY) broth (5 g of peptone, 3 g of yeast extract per liter; Difco, Detroit, Mich.) was employed as the mating medium. Additional matings were conducted as mixtures in PY broth, PY broth plus phage-specific antiserum, or on PY agar or Trypticase mating medium (2) using standard mating procedures (1).

Recombinant selection and scoring. The cells from the U-tube and PY broth crosses were harvested by centrifugation after 36 h of incubation in a shaking incubator at 30 C, washed with saline, and suspended in saline containing 10% (vol/vol) φEC-specific antiserum. The antiserum was produced in rabbits and possessed a titer with an average of K = 21/min. The cells were incubated in the antiserum for 15 min, centrifuged, and resuspended in saline. The cell suspensions were plated on minimal medium (MM) plates (1) supplemented for selection of the individual parental phenotypes and on PY plates to determine total cell density. Additional

samples were plated on MM and supplemented MM for recombinant selection. To score for lysogeny, plates containing the isolated parental types were replicated to PY plates and PY plates covered with a soft-agar overlay containing a sensitive indicator strain. After 12 h of incubation, the lysogens produced a clear zone of lysis around the colony. To assure that the colonies represented lysogenic strains and were not merely contaminated with phage, sample colonies were selected and cultured in PY broth for 48 h. At 3-, 6-, 12-, 24-, and 36-h intervals, phage-specific antiserum was added to the cultures. The samples were subcultured and tested for phage production.

Growth obtained from agar-mated strains was harvested from the surface of the plates, suspended in saline, and treated in a manner similar to that used for U-tube crosses. Mixed cultures of normally incompatible strains were prepared and tested as above. Controls included individually cultured parental strains.

RESULTS AND DISCUSSION

The results obtained from standard PY agar plate crosses between compatible and incompatible strains are provided in Table 1. Incompatible nonlysogenic strains failed to acquire the phage-bearing trait after their mixed growth with lysogenic strains. For example, the lysogen Ce3-53 (φEC) was grown in mixed culture with a nonlysogenic, arginine- and tryptophan-requiring strain (Ce3-90A). When the mixture was plated on MM plus arginine and tryptophan, no lysogenic colonies were observed among 2,500 replicated colonies (Table 1). That such results were not locus dependent was shown in the attempted mating of Ce3-69 (φEC), a lysogenic adenine-, phenylalanine-requiring strain, with Ce3-60, a nonlysogenic adenine-, arginine-requiring strain. Compatible combinations (Ce3 by cE2) produced 16 to 19% of the nonlysogenic parental phenotypes as phage-bearing colonies.

Recombination between chromosomal traits occurred during mixed growth of compatible lysogens (Table 2). When cell suspensions of Ce3-53 (φEC) and cE2-13 parental types were plated on MM plus arginine (*purB2+ his-3+ purA1+* selection) and MM plus adenine (*his-3+ arg-3+* selection), recombinant recovery was 2.3×10^{-5} and 9.7×10^{-5}, respectively. An analysis of the recombinant class types suggested that the phage-bearing trait is linked to chromosomal determinants. Class types originating from *purB2+ his-3+ purA1+* selection were all lysogenic (Pφe, class type 1, Table 2), whereas selection of *his-3+ agr-3+* loci produced 78% nonlysogenic class types (classes 2 and 4). In the reciprocal cross (Ce3-53 by cE2-13 (φEC), the *purB2+ his-3+ purA1+* selection resulted in all nonlysogenic progeny (class 5), and the *his-3+ arg-3+* selection produced 55% nonlysogenic recombinants (class type 6). Since the *his-3+ arg-3+* selection requires a crossover in region II or III (see linkage model, Table 2), the results indicate that the φEC-prophage is linked between the His and Arg markers in both the cE2-13 (φEC) and Ce3-53 (φEC) lysogens. Matings of the Ce3-53 (φEC) lysogen produced a streptomycin-sensitive (Str-S) class type that was nonlysogenic (class 4), suggesting that the prophage is integrated to the left of the *strB2* locus (see linkage model, Table 2). Matings of the cE2-13 (φEC) strain produced a Str-R class type, of which 43% were lysogenic (classes 6 and 7), and a Str-S class that was all lysogenic (class 8). Thus, a crossover event in region III appears to segregate the prophage and places it between the *strB2+* and *arg-3+* loci (see linkage model).

PY broth crosses performed in U-tube chambers separated by membrane filters failed to yield recombinants for either chromosomal traits or the φEC-prophage. No lysogenic colonies were observed in an analysis of about 2×10^3 colonies of the cE2-13 parental phenotypes, nor did any colonies appear on the selective media when the cultures were plated at cell densities of about 10^8 colony-forming units (CFU) per ml. Recombinants were recovered from mixed broth cultures after selection on MM plus arginine or MM plus adenine, although they were considerably less frequent (about 7×10^{-7} per CFU) than agar-mated strains. Phage-bearing isolates that corresponded to the nonlysogenic parental phenotype were also less abundant (1.8%) than with agar-mated strains. The addition of 10% φEC-specific antiserum to the PY broth did not affect the recovery of either chromosomal nor lysogenic recombinants.

Free-phage titers obtained from PY broth crosses of cE2-13 (φEC) by Ce3-53 were 4.5×10^5

TABLE 1. *Plasmid recovery from agar crosses of nocardial lysogens*

Lysogenic strain	Nonlysogenic strain	Plasmid recovery among the selected nonlysogenic strain after mating[a]
Ce3-53 (φEC)	Ce3-90A	$<4 \times 10^{-4}$
Ce3-69 (φEC)	Ce3-60	$<3 \times 10^{-3}$
cE2-13 (φEC)	cE2	$<4 \times 10^{-4}$
Ce3-53 (φEC)	cE2-13	1.7×10^{-1}
cE2-13 (φEC)	Ce3-353	1.9×10^{-1}
cE2-13 (φEC)	Ce3-69	1.6×10^{-1}

[a] Recovery is expressed as lysogenic colonies per total colonies tested.

TABLE 2. *Recombinant phenotypes recovered from matings of nocardial lysogens*

Strains[a] crossed	Selected traits	Recombinant[b] recovery	Recombinant[c] phenotypes	Class type[d] frequency
Ce3-53 (ϕEC) × cE2-13	*purB2+ his-3+ purA1+*	2.3×10^{-5}	(1) Str-R Arg$^-$ (Pϕe)	100
	his-3+ arg-3+	9.7×10^{-5}	(2) Str-R Pur$^-$	75
			(3) Str-R Pur$^-$ (Pϕe)	22
			(4) Str-S Pur$^-$	3
cE2-13 (ϕEC) × Ce3-53	*purB2+ his-3+ purA1+*	1.8×10^{-5}	(5) Str-R Arg$^-$	100
	his-3+ arg-3+	1.1×10^{-6}	(6) Str-R Pur$^-$	55
			(7) Str-R Pur$^-$ (Pϕe)	43
			(8) Str-S Pur$^-$ (Pϕe)	2
Ce3-53 × cE2-13	*purB2+ his-3+ purA1+*	5.9×10^{-4}	(9) Str-R Arg$^-$	100
	his-3+ arg-3+	1.2×10^{-4}	(10) Str-R Pur$^-$	96
			(11) Str-S Pur$^-$	4

[a] Strain genotypes: Ce3-53(ϕEC), *purB2 his-3+ purA1+ strB2 arg-3 (pϕe-1); cE2-13, purB2+ his-3 purA1 strB2+ arg-3+; Ce3-53, purB2 his-3+ purA1+ str-B2 arg-3;* cE2-13 (ϕEC), *purB2+ his-3 purA1 strB2+ arg-3+ (pϕe-6).*

[b] Recombinant recovery expressed as recombinants per total CFU.

[c] The recombinant phenotypes are numbered (1), (2), etc. for reference in text. R, Resistant; S, sensitive; +, synthesized; −, required; Arg, arginine; His, histidine; Pur, purine; (Pϕe), lysogenic (ϕEC prophage); Str, streptomycin.

[d] Class type frequencies were calculated as the percentage of the total sable recombinant population tested.

Linkage Model

cE2-13	*purB2+*		*his-3*	*purA1*		*strB2+*		*arg-3+*
		I			II		III	
Ce3-53 (ϕEC)	*purB2*		*his-3+*	*purA1+*		*strB2*	(*pϕe-1*) (*pϕe-6*)	*arg-3*
cE2-13 (ϕEC)	*purB2+*		*his-3*	*purA1*		*strB2+*		*arg-3+*
		I			II		III	
Ce3-53	*purB2*		*his-3+*	*purA1+*		*strB2*		*arg-3*

(For a detailed linkage map see Adams, 1974 [3].)

to 6.5×10^5 plaque-forming units/ml after 36 h of incubation. The incompatible combination, cE2-13 (ϕEC) by cE2-13, produced free-phage titers of about 3.2×10^5 to 4.9×10^5 plaque-forming units/ml. Since zygotic induction should increase free-phage titers, it does not appear to result in nocardial matings. Ratios of parental types in the mixtures were determined after incubation by plating samples on media selective for the parental types. Crosses initiated with 1:1 parental cell ratios were compared after 36-h incubation in PY broth. The mating mixtures of cE2-13 and Ce3-53 (ϕEC) produced parental cell ratios of about 1 cE2:155 (±73) Ce3 CFU. Reciprocal matings resulted in a ratio of 1 Ce3:105 (±90) cE2 parental cells. Thus, mortality of the nonlysogenic parental cells shifts the cell ratio in favor of the lysogenic parental type. This may account for the lower recombinant recovery obtained from matings of nocardial lysogens compared with nonlysogenic strains (Table 2).

Our results indicate that the ϕEC prophage exists as a plasmid and as an integrated element linked near the *arg-3*/*arg-3+* alleles. The high incidence of ϕEC inheritance suggests that matings between compatible cells are much higher than recombinant recovery would predict. For example, recombinant recovery from selection of chromosomal genes ranges from about 1.0×10^{-4} to 1.0×10^{-6}, whereas matings leading to transfer of the ϕEC-prophage occurred in about 17 to 19% of the population.

The majority of the mating events result in the transfer of the ϕEC prophage without any evidence of an interaction between chromosomal genes. However, this plasmid-like behavior of the prophage inheritance is absent when matings involve the reassortment of chromosomal traits. For example, 16 to 19% of the nonlysogenic population was found to have inherited the ϕEC prophage, whereas 100% of a specific recombinant class type either inherited

the prophage or failed to (classes 1 and 5). If the prophage exists as both an integrated element and plasmid in the same cell, then there appears to be competition between plasmid and chromosome during the mating event. If the prophage exists as either a plasmid or an integrated element, but not both in the same cell, then only those cells with an integrated prophage would reassort chromosomal genes. The latter is reminiscent of the *Escherichia coli* system. In any case, these lysogens should prove very useful for elucidating the mechanism(s) of nocardial recombination.

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