SHORT COMMUNICATIONS

Chromosomal Location of the ϕ C-Prophage in *Nocardia erythropolis*

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INTRODUCTION

The mating type strains of *Nocardia erythropolis* (Mat-Ce3 and Mat-cE2) are differentially infected by nocardiphage ϕ C, while both strains are susceptible to infection by a closely related phage ϕ EC. The phages were isolated from soil and characterized by Brownell, Adams & Bradley (1967). Crockett & Brownell (1972) isolated a Mat-Ce3 mating strain lysogenic for phage ϕ EC and later Brownell & Clark (1974) isolated a ϕ C lysogen of Mat-Ce3. In crosses, the ϕ EC-prophage behaved as a plasmid (Brownell, 1973).

This communication provides evidence for a chromosomal location of the factors affecting the inheritance of the ϕ C-bearing trait.

METHODS

Lysogenic strains were isolated as described by Crockett and Brownell (1972). Strain Mat-Ce3-90(ϕ C) requires adenine, tryptophan and arginine for growth and is resistant to 200 μ g streptomycin/ml and 10 μ g tetracycline/ml. Mat-cE2-91 requires histidine and pyridoxine for growth and is sensitive to both streptomycin and tetracycline. Standard nocardial mating techniques were used (Adams, 1964). The appropriate parental strains were grown in mixed culture on nutrient agar (Difco), and individually, as controls. Cultures were incubated for 48 to 72 h at 30 °C and saline suspensions were prepared from cross and control plates. In some cases, the cultures were treated with phage-specific antiserum for 3 h, centrifuged and washed in saline. The population density of saline suspensions was determined by plating on nutrient agar, while parental type ratios were obtained by plating on the appropriately supplemented minimal medium (SMM) plates. Recombinants were selected by plating samples on SMM or minimal medium (MM) plates. After 5 to 6 days' incubation, recombinant colonies were picked on to fresh plates, incubated for 24 h and replicated on to peptone-yeast extract (PY) plates (5 g peptone, 3 g yeast extract, Difco); inhibitor-containing minimal medium (IMM); SMM; and MM plates. The IMM, SMM and MM plates were incubated for 3 to 5 days and scored for recombinants. To test for lysogeny, the recombinants were replicated on to PY plates with soft-agar overlays containing a sensitive indicator strain, and examined for zones of lysis after 12 h incubation. The accuracy of this technique for scoring lysogeny was further tested by selecting about 250 phage-producing recombinants and culturing them in PY broth. At 0, 6, 12 and 24 h intervals, antiserum was added and they were retested for phage production.

RESULTS AND DISCUSSION

Crosses were conducted between both lysogenic and non-lysogenic strains in order to compare the segregation of unselected markers (Table 1). The recombinant recovery rates and class types prefixed (A) represent matings between non-lysogenic strains while those prefixed (B) are the results obtained from matings of Mat-Ce3-90(ϕ C) lysogens and non-lysogenic Mat-cE2-91 strains. A linkage model depicting the zygote is provided at the bottom of the table. No recombinants were recovered when about 1.0×10^8 colony forming units (c.f.u.)/ml were plated on MM for selection of prototrophic recombinants involving three or more markers. Recovery of recombinants selected for the Ad⁺ His⁺ phenotype averaged 1.9×10^{-4} from matings of non-lysogenic strains and 4.3×10^{-4} from crosses of lysogenic parental strains (Table 1). More than 90 % of the recombinant population produced from crosses of non-lysogenic, and lysogenic, strains were phenotypically Tet-R Pdx⁺ Try⁻ Str-R Arg⁻ (classes A1, B1; Table 1) and originated from a single crossover in region I (see linkage model; Table 1). Tetracycline-sensitive (Tet-S) classes (A2, B2) originated from single crossovers in region II while the rare streptomycin-sensitive class (A3, B3) required multiple crossovers in regions I, V and VI. All the recombinant classes from the Ad⁺ His⁺ selection were non-lysogenic (Lyc⁺).

Selection of the His⁺ Arg⁺ characters provided an unselective analysis of the Ad⁻ trait. The non-lysogenic parental types produced 88 % Ad⁻ Tet-R (class A4), 9 % Ad⁺ Tet-R (A5) and 1.5 % Ad⁺ Tet-S (A6). In the lysogenic cross, the Ad⁻ class was lysogenic (B4) and the Ad⁺ classes non-lysogenic (B5, B6). Thus, the phage-bearing trait segregated with the Ad⁻ marker. The Ad⁺ Pdx⁺ selection produced class types similar to the Ad⁺ His⁺ selection due to linkage of the His⁻ Pdx⁻ traits on the Mat-cE2-91 chromosome. Only 0.5 % of the recombinants analysed required histidine (class A9) indicating that the crossover region III is quite small. The recombination classes from matings with the lysogenic parental strain were non-lysogenic (B7, B8). The classes recovered from Arg⁺ Pdx⁺ resembled the phenotypes obtained from Arg⁺ His⁺ selection. Again the Ad⁻ classes were lysogenic (B9) while the Ad⁺ class was non-lysogenic (B10). The His⁺ Try⁺ selection produced primarily heterogenotes which segregated out Try⁻ class types during unselected growth. Only 8 to 10 % of the colonies produced on the selective medium were stable after subculturing, 7 to 9 % were Pdx⁻ (A12, B11) and 1 to 2 % Pdx⁺ (A13, B12). They resulted from single crossovers in regions III and IV respectively. Both class types obtained from crosses with the lysogen were phenotypically Ad⁻ Lyc⁻. Selection of Pdx⁺ Try⁺ produced all heterogenotes which segregated the Mat-Ce3-90 or Mat-Ce3-90(ϕ C) parental types (results not shown). Finally less than 3 % of the recombinant population selected for the Ad⁺ Tet-R phenotype was non-lysogenic (B14) suggesting close linkage of the ϕ C-prophage to the left of the Ad⁻ character.

Since Brownell & Clark (1974) had reported that the phage produced by the Mat-Ce3-90(ϕ C) lysogens could infect the Mat-cE2 mating strains, crosses were analysed for the effects of mixed growth on the Mat-cE2 parental strains. Crosses between non-lysogenic strains initiated with 1:1 parental cell ratios were 1:3 (± 2.5) Mat-cE2 to Mat-Ce3 after 48 h incubation. Matings of the lysogenic Mat-Ce3 produced cell ratios of 1:5 (± 4.5) Mat-Ce3-90(ϕ C) to Mat-cE2 and no significant mortality occurred due to infection of the Mat-cE2 strain by exogenous phage.

Suspensions of the mating mixtures were also treated with phage-specific antiserum and plated on SMM to select for the Mat-cE2 parental type. The SMM plates were replicated on to PY plates seeded with a phage-sensitive strain to determine if any lysogens were

Table 1. Segregation of unselected markers in recombinants obtained from matings of nocardial lysogens

Selected phenotypes	Recombinant recovery*	Class type†	Recombinant phenotype‡	Percentage of population§
Ad ⁺ His ⁺	(A) 1.9×10^{-4}	A1	Tet-R Pdx ⁺ Try ⁻ Str-R Arg ⁻	96.0
		A2	Tet-S Pdx ⁺ Try ⁻ Str-R Arg ⁻	2.7
		A3	Tet-R Pdx ⁺ Try ⁻ Str-S Arg ⁻	0.9
	(B) 4.3×10^{-4}	B1	Tet-R Pdx ⁺ Try ⁻ Str-R Arg ⁻ Lyc ⁺	91.3
		B2	Tet-S Pdx ⁺ Try ⁻ Str-R Arg ⁻ Lyc ⁺	7.1
		B3	Tet-R Pdx ⁺ Try ⁻ Str-S Arg ⁻ Lyc ⁺	0.6
His ⁺ Arg ⁺	(A) 7.3×10^{-4}	A4	Ad ⁻ Tet-R Pdx ⁺ Try ⁻ Str-R	88.3
		A5	Ad ⁺ Tet-R Pdx ⁺ Try ⁻ Str-R	9.1
		A6	Ad ⁺ Tet-S Pdx ⁺ Try ⁻ Str-R	1.5
	(B) 1.1×10^{-4}	B4	Ad ⁻ Tet-R Pdx ⁺ Try ⁻ Str-R Lyc ⁻	90.2
		B5	Ad ⁺ Tet-R Pdx ⁺ Try ⁻ Str-R Lyc ⁺	7.5
		B6	Ad ⁺ Tet-S Pdx ⁺ Try ⁻ Str-R Lyc ⁺	1.9
Ad ⁺ Pdx ⁺	(A) 3.1×10^{-4}	A7	Tet-R His ⁺ Try ⁻ Str-R Arg ⁻	95.1
		A8	Tet-S His ⁺ Try ⁻ Str-R Arg ⁻	3.2
		A9	Tet-S His ⁻ Try ⁻ Str-R Arg ⁻	0.5
	(B) 2.9×10^{-4}	B7	Tet-R His ⁺ Try ⁻ Str-R Arg ⁻ Lyc ⁺	95.8
		B8	Tet-S His ⁺ Try ⁻ Str-R Arg ⁻ Lyc ⁺	3.6
Arg ⁺ Pdx ⁺	(A) 5.4×10^{-4}	A10	Ad ⁻ Tet-R His ⁺ Try ⁻ Str-R	93.0
		A11	Ad ⁺ Tet-R His ⁺ Try ⁻ Str-R	6.4
	(B) 2.2×10^{-4}	B9	Ad ⁻ Tet-R His ⁺ Try ⁻ Str-R Lyc ⁻	94.2
		B10	Ad ⁺ Tet-R His ⁺ Try ⁻ Str-R Lyc ⁺	5.1
His ⁺ Try ⁺	(A) 5.5×10^{-4}	A12	Ad ⁻ Tet-R Pdx ⁻ Str-S Arg ⁺	7.2
		A13	Ad ⁻ Tet-R Pdx ⁻ Str-S Arg ⁺	1.2
	(B) 6.4×10^{-4}	B11	Ad ⁻ Tet-R Pdx ⁻ Str-S Arg ⁺ Lyc ⁻	9.3
		B12	Ad ⁻ Tet-R Pdx ⁺ Str-S Arg ⁺ Lyc ⁻	0.7
Ad ⁺ Tet-R	(A) 4.6×10^{-5}	A12	His ⁺ Pdx ⁺ Try ⁻ Str-R ⁻ Arg ⁻	97.4
	(B) 1.7×10^{-5}	B13	His ⁺ Pdx ⁺ Try ⁻ Str-R Arg ⁻ Lyc ⁺	96.1
		B14	His ⁺ Pdx ⁺ Try ⁻ Str-R Arg ⁻ Lyc ⁻	2.9

Linkage model

Ad ⁺ (Lyc ⁺)	Tet-S	His ⁻	Pdx ⁻	Try ⁺	Str-S	Arg ⁺
I	II	III	IV	V	VI	
Ad ⁻ (Lyc ⁻)	Tet-R	His ⁺	Pdx ⁺	Try ⁻	Str-R	Arg ⁻

* Recombinant recovery is expressed as the number of colonies observed on the selective media/total colony forming units plated per ml. (A), Recombinant recovery observed from matings of non-lysogenic strains; (B), recombinant recovery obtained from crosses of Mat-Ce3-90(ϕ C) lysogens and non-lysogenic Mat-cE2-91 strains.

† Class type references: A, class types obtained from crosses on non-lysogenic strains; B, class types observed from crosses of lysogenic strains.

‡ Symbols: +, synthesized; -, required; R, resistant; S, sensitive; Ad, adenine; Arg, arginine; His, histidine; Lyc, lysogenic for phage ϕ C; Pdx, pyridoxine; Try, tryptophan; Str, streptomycin; Tet, tetracycline.

§ Percentage of the recombinant population analysed.

produced. Out of about 5.0×10^3 colonies tested, no phage producing colonies of the Mat-cE2 phenotype were observed. Thus, unlike the ϕ EC-prophage, the ϕ C-prophage is inherited only in conjunction with chromosomal genes.

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