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NOTES

Genome Structure in *Streptomyces* spp.: Adjacent Genes on the *S. coelicolor* A3(2) Linkage Map Have Cotransducible Analogs in *S. venezuelae*

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Cotransduction analysis in *Streptomyces venezuelae* with the generalized transducing phage SV1 showed that several pairs of likely analogs of markers that are adjacent on the conjugational linkage map of *Streptomyces coelicolor* A3(2) were cotransducible and therefore physically close together. This supports the contention that taxonomically distinct "species" of *Streptomyces* are genetically closely related.

The gram-positive bacterial genus *Streptomyces* embraces over 400 "species," although the taxonomic status of perhaps two-thirds of these, based on numerical taxonomy, is uncertain (23). Data on genome structure derived by conventional genetic mapping and molecular cloning should provide more direct evidence of relationships among the streptomycetes as they have for enteric bacteria (14, 19). In extending to other species the well-developed genetic analysis of *Streptomyces coelicolor* A3(2) (8), Friend and Hopwood (5) drew attention to overall similarities in the relative locations of analogous markers on the chromosomal linkage maps of *S. coelicolor* A3(2) and *Streptomyces rimosus* despite the taxonomic separation of these species. [By extrapolation from its close relative *Streptomyces lividans*, *S. coelicolor* A3(2) is presumably a member of the *Streptomyces griseoruber* cluster in group A (cluster 21) of Williams et al. (23), while *S. rimosus* is in group B, cluster 42.] Similar comparisons with *S. coelicolor* A3(2) have been made in several other studies on conjugational linkage in *Streptomyces*. Those involving at least 10 markers are listed in Table 1.

Assuming that phenotypically similar markers in similar locations define analogous (or possibly homologous) genes, a review of the maps cited in Table 1 revealed that none of the other maps could be oriented to give total agreement with the *S. coelicolor* map, although *S. chrysomallus*, *S. rimosus* and *S. venezuelae* came very close and differed from *S. coelicolor* only in the relative orientations of three pairs of loci (*S. chrysomallus*) or the locations of *lysB* and *thr-4* (*S. rimosus*) or *lysA* (*S. venezuelae* and all three other strains in which this theoretically unique locus [4] was mapped). The present experiments were conducted to test the view (23) that most *Streptomyces* species are more closely related than existing taxonomic divisions would suggest. Since other closely related bacterial species have considerable similarities in their genetic organization (19), we reasoned that despite the present taxonomic gulf between *S. coelicolor* A3(2) and *S. venezuelae*, apparent analogs of markers that are conjugationally adjacent in *S. coelicolor* might be cotransducible by phage SV1 in *S. venezuelae*.

Since any two cotransducible markers must be physically closer together than the normal genome length of the transducing phage, which for SV1 is estimated to be about 45 kilobases (kb) (Stuttard and Dwyer, unpublished data), it follows that cotransduction analysis is an extremely reliable and efficient method of demonstrating the physical proximity of different genes. A fairly recent estimate puts the average *Streptomyces* genome length at about 6.75×10^3 kb (or 1.5 times larger than that of *Escherichia coli*) (7), although an earlier estimate was some 50% larger than this. Using either estimate, SV1-cotransducible genes can be no more than about 0.67% of the total *S. venezuelae* genome length apart. Transduction methods are described in an accompanying paper (22), and Table 2 lists the results of cotransduction tests for seven pairs of chromosomal markers. Data for cotransduction of *thrABC-lysA*, *hisAB-trpABC*, *argAB–cys-28*, and *cys-28–cml–pdxB* have already been published (20–22). Except for the *thr-lysA* linkage (discussed in reference 4), each pair of cotransducible markers (excluding the *cml* genes) has a directly comparable pair of adjacent (or, for *cys-28–pdx* and *rib-2–his-1*, potentially adjacent) analogs on the linkage map of *S. coelicolor* A3(2). However, we did not observe cotransduction between some pairs of markers which, on the basis of conjugational mapping and by analogy with *S. coelicolor*, seemed likely to be close together; thus, one putative *cysB* marker (*cys-13*) was not cotransducible with a putative *metB* marker (*met-13*); *gyl* markers were not cotransducible with *argB*; and several of our *ade* markers were not cotransducible with either of two *gua* markers. These negative results may mean simply that we were using markers that defined genes other than those that are adjacent in *S. coelicolor* or that similar recombinational intervals defined by heteroclone analysis in *S. coelicolor* (8) do not always define similar physical distances along the chromosome.

Our growing body of cotransduction data for *S. venezuelae* leads us to conclude that there is indeed very close genetic relatedness between *S. coelicolor* and *S. venezuelae* despite their taxonomic separation (Table 1) (23) and that the close recombinational proximity of many adjacent markers in two arcs of the *S. coelicolor* map, as determined by heteroclone analysis, is probably a reason-

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TABLE 1. Circular chromosomal linkage maps of *Streptomyces* species

Species (strain)	Numerical taxon[a]		Loci			Reference(s)
	G	C	Total no. mapped[b]	No. apparently concordant with *S. coelicolor*	Disconcordant types[c]	
S. coelicolor A3(2)	A?	21	126	126		10
S. achromogenes subsp. *rubradiris* (NRRL 3061)	A?	19	16	14	*met-1*	3
S. bikiniensis var. *zorbonensis*	A?	1B	15	12	*adeB, lysA, metB*	2
	B	64				
S. chrysomallus (ATCC 11523)	A?	1B	15	15	*(arg-1, ura-1), (pro-1, leu-1), (met-4, ade-1)*	12
S. clavuligerus (NRRL 3585)	J?	71	14	Unclear	Uncertain	13
S. glaucescens (ETH22794)	A?	28	23[d]	19	*arg-3, lys-1, -2, -3, met-2, thi-3*	11
S. griseus (NRRL 3851)	A?	1B	15	13	*ade-9, his-1, leu-2, rib-1*	16
S. lividans (66)	A	21	10	10	*(spc-1, str-6)*	9
S. olivaceus	A?	1C	35	29	*ade-2, arg-3, his-3, lys-1, lys-3, phe-1, rib-2, thi-1, tyr-1*[e]	15
	A	3				
S. rimosus (ATCC 10970)	B	42	22	19	*thr-4*	1, 17[f]
			26	22	*lysB, (adeA, uraA)*	5, 6, 18
S. venezuelae (ISP5230)	A	6	29	27	*lysA*	4, 22

[a] Taxonomic groups (G) and clusters (C) according to Williams et al. (23); question marks indicate uncertainty regarding the relationship between the strain used for mapping and the strain subjected to numerical classification.

[b] Includes markers for which current maps of *S. coelicolor* or the strain in question show no apparent analogs; thermosensitive markers are excluded; mapping was by conjugation, with heteroclone analysis done only in *S. coelicolor* A3(2) (8) and *S. rimosus* (1).

[c] Concordance was assessed by orientation of the respective maps to maximize the coincidence of ostensibly analogous loci, particularly with the *S. coelicolor* markers *hisA* (histidine but not histidinol utilization), *strA*, *proA*, *argA*, and *thrA*; antibiotic biosynthesis genes were excluded; gene designations of apparently disconcordant loci are those of the non-*S. coelicolor* strain; markers in parentheses are inverted relative to *S. coelicolor*.

[d] Different versions of the *S. glaucescens* map show different spectra of markers, making the overall total uncertain.

[e] Assuming *his-1* and *his-2* to be analogous to *hisA* and *hisD*, respectively, and *str-1* to be analogous to *strB* (low-level streptomycin resistance).

[f] Pigac and Alacevic, 1979; cited in Reference 17.

TABLE 2. SV1-cotransducible markers in *S. venezuelae*[a]

Markers	Approximate cotransduction frequency (%)
aro-1 uraB5	16
ilv-8 leuB7	8
leuA4 proA1	27
nicB8 trp-4	5
rib-2 his-1	39
rib-2 pdx-4,5	2, 4
rifA1 strA6	+[b]

[a] Previously published linkages are not included (see text); transduction methods are described in Vats et al. (22). Most crosses were done reciprocally, and up to 200 transductants were characterized from each cross.

[b] Measurement of cotransduction frequency was not possible because all transductants were unselected; instead, an Scp^r Rif^s Ura$^-$ Lys$^-$ recombinant was recovered from a transduction involving an Scp^r Rif^r Ura$^-$ Lys$^-$ recipient, and phage grown on an Scp^r Rif^s His$^-$ Ade$^-$ donor. This experiment was done by Stephen Allen.

ably accurate reflection of physical proximity. Unfortunately, SV1-mediated transduction analysis is presently only applicable to *S. venezuelae*, and there is still no generalized transducing phage available for *S. coelicolor* A3(2). Therefore, whether the discrepancies between the linkage maps of other *Streptomyces* spp. reflect real differences in genome organization or result from conjugational mapping artifacts remains to be determined, either by development of new generalized transduction systems or by other methods of fine-structure analysis, such as molecular cloning.

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