Conjugational Fertility and Location of Chloramphenicol Biosynthesis Genes on the Chromosomal Linkage Map of *Streptomyces venezuelae*

By J. L. DOULL,¹† S. VATS,² M. CHALICIOPOULOS,² C. STUTTARD,¹*
K. WONG¹‡ AND L. C. VINING²

Departments of Microbiology¹ and Biology², Dalhousie University, Halifax, Nova Scotia,
Canada B3H 4H7

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In *Streptomyces venezuelae* fertility, defined as chromosomal gene recombination, was enhanced over 1000-fold when one parent in a biparental conjugational cross lacked the physically-undetected plasmid SVP1, as compared with crosses in which both parents carried SVP1. The existence of SVP1 and at least two other fertility plasmids, SVP2 and SVP3, was detected in *S. venezuelae* by 'lethal zygotism' elicited by a plasmid-plus mycelium in contact with a plasmid-minus mycelium. Conjugational crosses were used to construct a linkage map of *S. venezuelae* which was highly consistent with the map of analogous loci in *S. coelicolor* A3(2). A cluster of genes governing chloramphenicol biosynthesis was located near *arg*, *cys* and *pdxB* genes at a position roughly equivalent to the 1–2 o'clock region of the *S. coelicolor* A3(2) map.

INTRODUCTION

In previous work with chloramphenicol-producing (Cml⁺) strains of *Streptomyces* we showed that physically detectable plasmids are not involved in the biosynthesis of this antibiotic (Ahmed & Vining, 1983; Doull *et al.*, 1983). To investigate further the genetic control of chloramphenicol biosynthesis in *S. venezuelae* ISP 5230 (the type strain of the species, also listed as ATCC 10712) we isolated and characterized 12 independent chloramphenicol-nonproducing (Cml⁻) mutants (Doull *et al.*, 1985). Owing to a lack of conjugational fertility in genetically marked derivatives of Cml⁺ and Cml⁻ strains we were initially unable to confirm an earlier report that genes encoding enzymes in the chloramphenicol biosynthetic pathway had a chromosomal location in derivatives of strain ISP 5230 (Akagawa *et al.*, 1975, 1979).

Since plasmid-determined conjugational fertility (detected as chromosomal gene recombination) is apparently widespread in *Streptomyces*, and recombinant frequencies are often higher when one parent in a cross lacks the fertility plasmid (plasmid-minus) while the other parent is plasmid-plus (Friend *et al.*, 1978; Bibb & Hopwood, 1981; Hopwood & Chater, 1984), it seemed likely that such fertility might be obtainable in *S. venezuelae*, either by elimination of a physically undetected endogenous fertility plasmid, or by introduction of an exogenous plasmid. Fertility plasmids are not always physically detectable by standard procedures, but seem to be almost universally detectable through 'pock' formation or 'lethal zygotism' (Ltz⁺) (Bibb *et al.*, 1981; Bibb & Hopwood, 1981; Hopwood & Chater, 1984). This phenotype is recognizable as a retardation of aerial mycelium growth (lethality being doubtful) and is observed after spores of a plasmid-plus (Ltz⁺) strain are spotted or replicated on a lawn of spores of a plasmid-minus ('lethal-zygotism-sensitive', Ltz^s) indicator strain (Bibb *et al.*, 1981; Hopwood *et al.*, 1983). Strains lacking plasmid are frequently isolated from among colonies of regenerated protoplasts

† Present address: Department of Microbiology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JG, UK.

‡ Present address: CIFT, Technical University of Nova Scotia, Halifax, Nova Scotia, Canada.

(Hopwood, 1981; Hopwood *et al.*, 1983), although they may also arise as spontaneous segregants in otherwise plasmid-plus spore populations (Friend *et al.*, 1978). They may be detected either by their *Ltz*^s phenotype when tested with parental spores or by their enhanced fertility in plate crosses (Bibb *et al.*, 1977; Friend *et al.*, 1978).

In this paper we report the isolation of conjugationally fertile *Ltz*^s derivatives of *S. venezuelae* strain ISP 5230 resulting from putative plasmid loss, and their use in the construction of a chromosomal linkage map that includes the *cml* loci defined by the mutations in our 12 *Cml*⁻ mutants.

METHODS

Strains, growth conditions and genetic techniques. Genetically marked strains were isolated after mutagenesis of the parental prototrophic chloramphenicol-producing type strain of *S. venezuelae*, ISP 5230 (also listed as ATCC 10712) (Stuttard 1982; Doull *et al.*, 1985). Auxotrophic mutants of SPI 5230 used in the present study are listed in Table 1. Gene designations are given to correspond with analogous loci in *S. coelicolor*, or to show differences, or are omitted when the phenotypic characterization is insufficient to define the gene. Agar media for growth of the bacteria have been described previously (Stuttard 1982; Doull *et al.*, 1985). In conjugational crosses about 10⁶ spores (c.f.u.) of a putative plasmid-plus strain were spread together with an excess (usually 20-fold) of spores of a putative plasmidless strain on MYM agar to give approximately equal frequencies of parental-type spores among the progeny, so balancing the cross. Analysis of crosses was done by either the 'four-on-four' or the single-selection (allele-gradient) procedure of Hopwood (1967); these procedures are described in Hopwood *et al.* (1985). All incubations were at 30 °C.

In the 'four-on-four' procedure, spores from a mixed culture of two mutants differing at four loci were spread on four different types of selective agar plates on which parental-type spores would not grow. The phenotypes of a sample of recombinants appearing on each medium were determined by replication to diagnostic media which allowed the nine recoverable (of 14 possible) recombinant phenotypes (including two reciprocal pairs) to be identified. As Hopwood (1967, 1972; Hopwood *et al.*, 1985) has pointed out, the validity of subsequent analysis depends upon the data satisfying two internal conditions: (a) to exclude the possibility of bias against the growth of any recombinant with a particular phenotype, the frequency (c.f.u. per unit volume of progeny spores at a standard dilution) of each recombinant type should be about the same on at least two of the four selective media; (b) both members of the two pairs of complementary genotypes that can be recovered should arise at approximately equal frequencies to indicate statistically equal (non-polarized) contributions by the two parents to the recombinant genomes. If there were greater than fivefold differences in any of these internal checks the data were not used in our analysis.

When the relative locations of selective markers had been established on a circular map in 'four-on-four' crosses, the location of any *cml* marker present was then determined as follows. Recombinant progeny from crosses between chloramphenicol-producing and non-producing mutants were picked from selective plates and restreaked on MYM agar to obtain single colonies. After sporulation, single colonies were patched on fresh selective agar, grown to sporulation and replicated to diagnostic media for marker verification. Inocula were then taken from each master patch and tested for chloramphenicol production by bioassay (see below).

When pairs of mutually fertile strains with five or six different markers of known relative locations were available, a new marker in either strain could be located by the 'allele-gradient' procedure (Hopwood, 1967, 1972; Hopwood *et al.*, 1985): two well-separated markers, one from each parent, were chosen as selective markers, all others being non-selective. Recombinants obtained from the cross after plating progeny spores on a selective medium were phenotypically characterized as in 'four-on-four' crosses. The frequency of each member of each pair of unselected alleles in the cross was then estimated. These 'allele ratios' formed clockwise and anticlockwise gradients on either side of each selective marker: parental-type alleles close to the selected parental marker were more frequent in recombinants than those further away. The allele ratio of the new marker was used to locate that marker in either the clockwise or anticlockwise gradient. The final position chosen was that which minimized the number of multiple cross-over progeny from the cross.

Isolation of fertility variants. The technique used initially was essentially that of Hopwood *et al.* (1983). Protoplast regenerants of auxotrophically-marked strains were patched to MYM, spotted with spores of their parental strain, incubated for 3–5 d, and then screened for a 'lethal-zygosis' (*Ltz*^s) response. Subsequent screenings were done with patches made directly from single colonies grown on MYM or supplemented minimal agar from various spore suspensions (either individual stock cultures or individual progeny from crosses). Putative plasmid-minus isolates were then tested for fertility (chromosomal gene recombination) in plate crosses with a differently marked '*Ltz*⁺' strain. The parental '*Ltz*⁺' strain was used as a control on the same plates (Friend *et al.*, 1978).

Screening for chloramphenicol production. Spores from the colonies to be tested were spread in patches in MYM agar (five equally spaced patches per 85 mm diameter plate). The patched colonies were grown for 48–72 h; the

Table 1. *Streptomyces venezuelae* strains

VS Strain no.	Chromosomal markers*	Source† or reference
65	<i>his-6</i>	Stuttard (1983b)
88	<i>metA8</i>	Spontaneous reversion of <i>his-5</i> in a double mutant (VS104) (C. Stuttard, unpublished data)
98	<i>arg-6 cml-4</i>	Cml-4 (MOP)
113	<i>his-6 adeA10</i>	VS65 (MOP)
130	<i>cysB13 cml-4</i>	Cml-4 (MOP)
141	<i>lysA7 cml-1</i>	Stuttard (1983a)
143	<i>metB14 cml-7</i>	Cml-7 (UV, Z. Ahmed)
146	<i>nicB6 cml-7</i>	Cml-7 (MOP, Z. Ahmed)
153	<i>trp-3 cml-5</i>	Stuttard (1983b)
160	<i>uraA1 thrC1 trp-5</i>	VS83 (Stuttard, 1983a) by UV mutagenesis (Z. Ahmed)
161	<i>uraA1 thrC1 tyr-2</i>	VS83 (UV, Z. Ahmed)
162	<i>uraA1 thrC1 ilv-5</i>	VS83 (UV, Z. Ahmed)
166	<i>uraA1 lysA1</i>	Transduction VS83(×)VS1 (Stuttard, 1983a)
191	<i>his-6 adeA10</i>	This paper
192	<i>his-6 adeA10</i>	This paper
194	<i>his-6 adeA10</i>	This paper
206	<i>his-6 adeA10 strA6</i>	Spontaneous, from VS191
227	<i>uraB5 cml-2</i>	Cml-2
230	<i>proA1 cml-6</i>	Cml-6
237	<i>lysA10 cml-6</i>	Cml-6
242	<i>rib-2 cml-6</i>	Cml-6
246	<i>pheA6 cml-11</i>	Cml-11
251	<i>nicB8 cml-11</i>	Cml-11
253	<i>pdxB3 cml-11</i>	Cml-11
263	<i>cys-28 cml-12</i>	Cml-12
296	<i>adeA10 uraA1 thrC1 trp-5 strA6 rifA3</i>	Spontaneous Rif ^R mutant of VS294, a recombinant from VS206 × VS160
297	<i>metA8 strA6</i>	'Ltz ⁻ ' recombinant from VS206 × VS88
300	<i>adeA10 tyr-2 strA6</i>	'Ltz ⁻ ' recombinant from VS206 × VS161
304	<i>arg-6</i>	'Ltz ⁻ ' recombinant from VS300 × VS98
305	<i>adeA10 uraA1 thrC1 ilv-5 strA6</i>	Recombinant from VS206 × VS162
306	<i>uraA1 thrC1 ilv-5</i>	'Ltz ⁻ ' recombinant from VS206 × VS162
310	<i>adeA10 cysB13 strA6</i>	Recombinant from VS206 × VS130

* Gross phenotypes of single auxotrophs as in Hopwood *et al.* (1985), except that *pdxB* gave a requirement for pyridoxal rather than pyridoxine; *cys-28* gave a *cysE* phenotype.

† Source strains were mutagenized with EMS (ethylmethane sulphonate) unless otherwise indicated: MOP, 8-methoxypsoralen plus near UV; UV, ultraviolet light, 256 nm. Cml⁻ mutants are described in Doull *et al.* (1985).

plates were then carefully overlaid between the patches with soft (0.5%, w/v) GNY agar seeded with *Micrococcus luteus*. The overlaid plates were incubated overnight and inhibition zones were examined. Wild-type chloramphenicol producers gave a clear zone extending about 10–15 mm from the edge of the patch.

RESULTS

Evidence for three conjugative plasmids in S. venezuelae

The doubly auxotrophic strain VS113 (*his-6 adeA10*) was tested for the occurrence of Ltz^s variants after protoplast formation and regeneration. Of 100 regenerants spotted with a suspension of VS113 spores (10⁷ c.f.u. ml⁻¹), two patches showed zones of aerial mycelium growth retardation at the periphery of the VS113 spot. These zones were phenotypically different (Fig. 1): a more distinct zone (viewed by transmitted light) was elicited on the regenerant designated VS191, and a less distinct zone on VS192. Similar, reciprocal Ltz tests between VS191 and VS192 showed that each elicited a different retardation zone when spotted on a patch of the other, but not on themselves or on the parent, VS113. A further variant, VS194, was isolated from strain VS191 after protoplast formation, regeneration and Ltz testing. Zones

Fig. 1. Test for 'lethal zygotis' (light areas in or around spots) among derivatives of strain VS113, viewed by transmitted light.

of aerial mycelium growth retardation were elicited on patches of strain VS194 by spores of VS113 and the related strains VS191 and VS192. In addition, strain VS194 elicited a faint Ltz^+ response on VS192 but not on VS191 or VS113. The derivations and Ltz responses of VS113 and its variants, and their interpretation using putative conjugative plasmid effects are given in Fig. 2. The proposed interpretation is based on that given by Hopwood *et al.* (1983) for similar results obtained with *S. lividans*. Thus, it would appear that there are at least three different conjugative plasmids in strain VS113 (and presumably in its parent strain ISP 5230). Although none of these was physically demonstrable by standard techniques (Doull *et al.*, 1983; Ahmed & Vining, 1983), in conjugational crosses involving VS191 or other Ltz^s strains (see below) more than 90% of recombinant progeny, and 78–97% of progeny with the chromosomal genotype of the Ltz^s parent, were converted to an Ltz^+Ltz^R phenotype (Table 2). The results were consistent with high-frequency transfer of fertility plasmids. In conformity with established nomenclature for similar fertility plasmids in other streptomycetes, we propose to call these plasmids SVP1, SVP2, and SVP3 (Fig. 2).

The possibility that ' Ltz^s ' segregants might arise spontaneously rather than through the process of protoplast formation and regeneration was investigated by screening 100 single colonies of each of several different auxotrophic derivatives of the wild-type strain of ISP 5230. Of the eight spore stocks examined, five (including VS113) showed 1–2% ' Ltz^s ' variants, mostly of the VS192 type (SVP3⁻). Therefore, it is likely that protoplast formation and regeneration were not essential for the recovery of VS191 and VS192 from VS113, although elimination of SVP2 (to give VS194) may have required this treatment.

Fig. 2. Putative plasmid content and 'Ltz' responses of VS113 and its derivatives. Solid arrows indicate strain derivations by plasmid loss or, for VS206, by spontaneous mutation. Open arrows indicate 'lethal zygosis' elicited by the designated plasmid(s) in the plasmid-plus strain on contact with the corresponding plasmid-minus mycelium.

Table 2. Effects of plasmid on chromosomal gene recombination, and evidence for 'infectious' transfer of plasmid *SVP1* in 'four-on-four' crosses involving strain VS113 (*his-6 ade10*) and its putative plasmid-minus derivatives

Parental strains* (and plasmid deficiencies)	Recombinant frequency† (per total progeny c.f.u.)	Progeny tested		
		Type‡	No.	% <i>SVP1</i> ⁻
VS113 × VS166	<10 ⁻⁷	—	0	—
VS191 (<i>SVP1</i> ⁻) × VS166	10 ⁻⁴	VS166	146	<1
		VS191	50	22
		Rec	483	1
VS192 (<i>SVP3</i> ⁻) × VS166	10 ⁻⁶	—	0	—
VS194 (<i>SVP1</i> ⁻ <i>SVP2</i> ⁻) × VS166	2.5 × 10 ⁻⁴	—	0	—
VS206 (<i>SVP1</i> ⁻) × VS88	10 ⁻⁴	Rec	289	3.5
VS206 (<i>SVP1</i> ⁻) × VS98	10 ⁻⁴	Rec	493	5
VS206 (<i>SVP1</i> ⁻) × VS161	10 ⁻⁴	Rec	305	7.5
VS300 (<i>SVP1</i> ⁻) × VS98	10 ⁻⁴	VS300	190	2.6
		VS98	239	0.8

* See Table 1 for genotypes and Fig. 2 for plasmids; plasmid-plus parental spores were mixed with a 20-fold excess of plasmid-minus parental spores to give approximately equal frequencies of parental-type c.f.u. in the progeny from each cross on MYM.

† Recombinant totals were obtained from the four different selective agar plates; c.f.u. were counted on MYM. Reversion frequencies for each marker in all crosses were in the range 10⁻⁷–10⁻⁹ per c.f.u.

‡ Strain designations indicate parental-type progeny; Rec, recombinant progeny of all selected genotypes.

When strains VS113, VS191 (*SVP1*⁻), VS192 (*SVP3*⁻) and VS194 (*SVP1*⁻*SVP2*⁻) were tested for chloramphenicol production after growth for 7 d in production medium (Doull *et al.*, 1985) the concentrations obtained were approximately 170, 130, 130, and 120 µg ml⁻¹, respectively. Thus loss of none of the plasmids had any significant effect on chloramphenicol biosynthesis.

The relative fertilities of strain VS113 and its derivatives were tested simultaneously in a plate cross with strain VS160 (*uraA1 thrC1 trp-5*). The result (not shown) was that VS191 and VS194 gave similar frequencies of recombinant colonies, and these were visibly higher than that given by VS192, while VS113 gave no recombinants with VS160 in this test. Therefore, it appeared that *SVP1* had a major role in recombinant formation and functioned like *SCP2* and *SLP2* in *S. lividans* 66 (Hopwood *et al.*, 1983), or *SRP1* in *S. rimosus* (Friend *et al.*, 1978). Likewise, *SVP3* mediated recombination but at a much lower frequency than *SVP1*. More precise estimates of

Fig. 3. Alternative arrangements of markers in the cross VS206 $\times$ VS237. The frequencies shown are the mean relative frequency of reciprocal recombinants requiring quadruple crossovers.

Table 4. Chloramphenicol production in recombinant progeny from cross VS206 (*his-6 adeA10 strA6*) $\times$ VS237 (*lysA10 cml-6*)

Recombinants				Inferred location of <i>cml-6</i> (see Fig. 5)
Genotype	Mean frequency*	No. tested	% <i>cml</i> ⁺	
+ + + <i>str</i>	33	70	2.8	Not between <i>his</i> and <i>adeA</i>
+ + + +	220	70	0	Not between <i>adeA</i> and <i>strA</i>
<i>his</i> + + <i>str</i>	7.5	20	100	Between <i>his</i> and <i>lysA</i> or <i>lysA</i> and <i>strA</i>
+ <i>ade</i> + <i>str</i>	80	70	8.5	Not between <i>adeA</i> and <i>lysA</i>
+ + <i>lys</i> <i>str</i>	100	70	0	Not near <i>strA</i> , possibly between <i>his</i> and <i>lys</i>
+ <i>ade</i> <i>lys</i> <i>str</i>	80	45	0	Not between <i>adeA</i> and <i>strA</i>
<i>his</i> + + +	30	4	100	Not near <i>strA</i>

Conclusion: the most likely location of *cml-6* is between *his* and *lysA*.

* Number of colonies per 0.1 ml of spore suspension diluted 10^{-2} and spread on selective media.

Table 5. Conclusions from 'four-on-four' crosses mostly between the *Cml*⁺ strain VS206 (*his-6 adeA10 strA6*) and *Cml*⁻ parents bearing single auxotrophic markers

Cml ⁻ Parent		Relative locations* of	
Strain no.	Genotype	Selective markers	<i>cml</i> mutations
VS98†	<i>arg-6 cml-4</i>	<i>arg-6</i> between <i>his-6</i> and <i>strA6</i>	—
VS130†	<i>cysB13 cml-4</i>	<i>cysB13</i> between <i>adeA10</i> and <i>strA6</i>	—
VS141	<i>lysA7 cml-1</i>	<i>lysA7</i> as <i>arg-6</i>	<i>cml-1</i> between <i>his-6</i> and <i>lysA7</i>
VS143	<i>metB14 cml-7</i>	<i>metB14</i> as <i>cysB13</i>	—
VS146	<i>nicB6 cml-7</i>	<i>nicB6</i> between <i>his-6</i> and <i>adeA10</i>	<i>cml-7</i> between <i>his-6</i> and <i>strA6</i>
VS153	<i>trp-3 cml-5</i>	<i>trp-3</i> very near <i>his-6</i>	<i>cml-5</i> between <i>trp-3</i> and <i>strA6</i>
VS166†‡	<i>uraA1 lysA1</i>	<i>uraA1</i> between <i>his-6</i> and <i>strA6</i> , opposite <i>lysA1</i>	—
VS227	<i>uraB5 cml-2</i>	<i>uraB5</i> as <i>arg-6</i>	<i>cml-2</i> near <i>uraB5</i>
VS230	<i>proA1 cml-6</i>	<i>proA1</i> as <i>nicB6</i>	<i>cml-6</i> between <i>his-6</i> and <i>strA6</i>
VS237	<i>lysA10 cml-6</i>	<i>lysA10</i> as <i>arg-6</i>	<i>cml-6</i> between <i>his-6</i> and <i>lysA10</i>
VS242	<i>rib-2 cml-6</i>	<i>rib-2</i> as <i>arg-6</i>	<i>cml-6</i> near <i>rib-2</i>
VS246	<i>pheA6 cml-11</i>	<i>pheA6</i> as <i>nicB6</i>	—
VS251	<i>nicB8 cml-11</i>	<i>nicB8</i> as <i>nicB6</i>	<i>cml-11</i> between <i>his6</i> and <i>strA6</i>
VS253	<i>pdxB3 cml-11</i>	<i>pdxB3</i> as <i>arg-6</i>	—
VS263	<i>cys-28 cml-12</i>	<i>cys-28</i> as <i>arg-6</i>	<i>cml-12</i> near <i>cys-28</i>

* On a circular linkage map.

† Both parents in the cross were *Cml*⁺.

‡ VS166 was crossed with VS191 (*his-6 adeA10*).

Fig. 4. Alternative locations of *ilv-5* in the cross VS206 × VS162.Table 6. Mapping *ilv-5* by single selection (*His⁺ Str^R*)Cross: VS206 (*his-6 ade10 strA6*) × VS162 (*uraA1 thrC1 ilv-5*)

Genotypes of selectable recombinants*				Numbers of:		
				Recombinant progeny	Crossovers required†	
					a	b
<i>adeA10</i>	+	+	+	220	2	2
+	<i>uraA1</i>	+	+	20	2	4
+	+	<i>thrC1</i>	+	0	6	4
+	+	+	<i>ilv-5</i>	1	4	4
<i>adeA10</i>	<i>uraA1</i>	+	+	15	2	4
<i>adeA10</i>	+	<i>thrC1</i>	+	0	4	2
<i>adeA10</i>	+	+	<i>ilv-5</i>	9	2	2
+	<i>uraA1</i>	<i>thrC1</i>	+	2	4	4
+	<i>uraA1</i>	+	<i>ilv-5</i>	10	2	2
+	+	<i>thrC1</i>	<i>ilv-5</i>	4	4	4
<i>adeA10</i>	<i>uraA1</i>	<i>thrC1</i>	+	0	4	4
<i>adeA10</i>	+	<i>thrC1</i>	<i>ilv-5</i>	25	2	2
<i>adeA10</i>	<i>uraA1</i>	+	<i>ilv-5</i>	1	2	2
+	<i>uraA1</i>	<i>thrC1</i>	<i>ilv-5</i>	73	2	2
<i>adeA10</i>	<i>uraA1</i>	<i>thrC1</i>	<i>ilv-5</i>	21	2	2
+	+	+	+	5	4	4
Total:				406		
Total recombinants requiring 4 or 6 crossovers:					12	47

* Selective markers are omitted.

† Alternative arrangements of markers: a, *ilv-5* between *his-6* and *thrC1*; b, *ilv-5* between *his-6* and *uraA1* (see Fig. 4).

same region of the *S. venezuelae* linkage map between *his-6* and *lysA*. In particular, markers *cml-2*, -6 and -12 are near markers that, by analogy with *S. coelicolor*, are probably in the 1-2 o'clock region of the linkage map.

Mapping by single selection

Multiply-marked strains bearing a new mutation together with mutations for which the relative order was known were used in further crosses. Each involved a single selection, with

Fig. 5. Linkage map of *S. venezuelae*. Spacing between loci is arbitrary except that those previously shown to be cotransducible are bracketed together. Relative orders of markers shown on inner and outer arcs have not been determined.

Table 7. Location of auxotrophic markers by single selection (allele-gradient analysis)

Cross*	Marker arrangement†
VS206 × VS161	<i>tyr-2</i> between <i>trp-5</i> and <i>thrC1</i>
VS206 × VS162	<i>ilv-5</i> between <i>his-6</i> and <i>thrC1</i>
VS296 × VS297	<i>metA8</i> between <i>trp-5</i> and <i>uraA1</i>
VS304 × VS305	<i>arg-6</i> between <i>ilv-5</i> and <i>uraA1</i>
VS306 × VS310	<i>cysB13</i> between <i>adeA10</i> and <i>strA6</i>

* See Table 1 for strain genotypes.

† On a circular linkage map: *thrC1* and *trp-5* were previously located by cotransduction very close to *lysA* and *his-6*, respectively (Stuttard, 1983*a*, *b*).

recombinants subjected to an 'allele-gradient' analysis from which the location of the new mutation was deduced (Hopwood, 1967; Hopwood *et al.*, 1985). Several crosses gave rise to putative heterokaryons (colonies giving no growth when spores were patched to fresh plates of the same selective agar) at frequencies of less than 5%. Heterokaryons were not considered in the present analysis. An example of single-selection analysis is given in Table 6 and Fig. 4. The conclusions drawn from five such crosses (Table 7) are incorporated with data obtained by 'four-on-four' analyses in the conjugational linkage map of *S. venezuelae* shown in Fig. 5.

DISCUSSION

The present results obtained with *S. venezuelae* are directly analogous to similar observations of fertility in *S. rimosus* (Friend *et al.*, 1978) and *S. lividans* 66 (Hopwood *et al.*, 1983) in that one or more physically undetected self-transmissible genetic elements, presumably plasmids, are involved. Also, it is apparently the loss of plasmid that generates the subsequent fertility of the plasmid-minus strains in crosses with a strain that has retained the plasmid. The range of markers in plasmid-minus strains is presently too limited to permit comprehensive estimates of relative fertilities mediated by each of the plasmids SVP1, SVP2 and SVP3 alone, and strains that lack all three plasmids have yet to be isolated. However, the present studies suggest that SVP1 is the major fertility plasmid in *S. venezuelae*, while SVP3 mediates a considerably lower frequency of chromosomal recombination. The role of SVP2 in fertility is not clear from the

present data, although it does seem to give a slight additive effect with SVP1 (compare the fertilities of VS191 and VS194 in Table 2).

The presence of all three plasmids in both parents in a mating mixture inhibited chromosomal recombination, but apparently did not block heterokaryon formation. Complementary plasmid-plus auxotrophs inoculated close together in patches on minimal agar often gave rise to tufts of growth in regions where the two patches met. However, only parental-type spores (in approximately equal frequencies) were recovered from such growth (C. Stuttard, unpublished data). The fact that hyphal contact was essential for this effect suggests that crossfeeding was not involved. Hyphal fusion and consequent heterokaryon formation would allow genetic complementation to give the prototrophic growth phenotype. A similar phenomenon has been documented in *S. coelicolor* A3(2) (Micheli *et al.*, 1984).

If, as seems likely, hyphal fusion and heterokaryon formation can occur in *Streptomyces* regardless of the plasmid status of parental strains, then the role of a 'fertility' plasmid may be in the activation of a chromosomal *recA*-type gene in the plasmid-minus host, thus facilitating chromosomal recombination within the heterokaryon. Alternatively, the plasmid may carry its own *recA*-type gene which becomes activated when the plasmid is introduced into a previously plasmidless cytoplasm. Obviously, the answers to these questions await the isolation of Rec⁻ strains.

In addition to establishing the presence of fertility factors in *S. venezuelae*, the present work complements our previous determination that plasmids are not involved in chloramphenicol biosynthesis (Ahmed & Vining, 1983; Doull *et al.*, 1983) by defining the chromosomal location of *cml* genes. Also, an association between the fertility factors and antibiotic production can be discounted from the fact that strains VS113, VS191, VS192 and VS194 all produce similar levels of chloramphenicol. Two previous studies (Francis *et al.*, 1975; Akagawa *et al.*, 1975) generated preliminary linkage maps of *S. venezuelae* strains 3022a(13s) and ISP 5230, respectively, but neither produced adequate data to locate *cml* mutations. In subsequent work, Akagawa *et al.* (1979) located *cpp* (*cml*) markers between 'met' and 'ilv' on a linkage map of *S. venezuelae* ISP 5230 which included six other chromosomal markers. Assuming that the 'met' and 'ilv' loci were analogous to *metA8* and *ilv-5* markers on the present map (Fig. 5), then the locations of *cpp* and *cml* markers are mutually consistent. However, Akagawa *et al.* (1975, 1979) did not isolate discrete fertility variants, and did not use the conventional 'four-on-four' and single-selection analyses in their mapping. More particularly, the location of a 'lys' marker between *met* and *leu* was deemed to be consistent with the similar location of a 'lys' marker on the *S. coelicolor* A3(2) linkage map. This is diametrically opposite to the location of ten independent *lys* mutations found in our present and previous work (Stuttard, 1983a; Fig. 5). There should be only one locus for uniquely lysineless mutations, namely *lysA*, which specifies the final enzyme in lysine biosynthesis, diaminopimelate decarboxylase (K. Wong & C. Stuttard, unpublished data). Any other mutant defective in lysine biosynthesis through an earlier block in the lysine pathway should also require diaminopimelate for growth and would be characterized as a *dap* mutant. When the linkage maps of other *Streptomyces* species, including *S. achromogenes* (Coats & Stroman, 1978) and *S. rimosus* (Rhodes *et al.*, 1981), are aligned with the *S. coelicolor* A3(2) map each has a single *lys* locus in the same relative position as *lysA* in Fig. 5. The apparently discrepant location of *lysA* in *S. coelicolor* A3(2) may have resulted from misclassification of a leaky *dap* mutant which was not biochemically characterized (D. A. Hopwood, personal communication). The relative location of a *pro* marker on the map of Akagawa *et al.* (1979) also differs from *pro* on the present *S. venezuelae* map; only the latter is consistent with the location of a single *pro* locus in *S. coelicolor* as well as in *S. lividans*, *S. glaucescens* and *S. rimosus*. A reorientation of markers on the Akagawa map could make the arrangement of most loci, including *lys* and *pro*, consistent with the present map, but would place *cpp* loci diametrically opposite to *cml*, and would leave *str* and *ade* in aberrant positions.

Since these discrepancies raise some doubts about the reliability of the unconventional mapping procedure used by Akagawa *et al.* (1975, 1979), we believe that the present data represent a more satisfactory determination of the chromosomal location of *cml* genes on a linkage map of *S. venezuelae*. This map is considerably more detailed than any previously

available, and is (almost) entirely consistent with the arrangement of analogous (if not homologous) loci in *S. coelicolor* A3(2). This relationship has also been confirmed in cotransduction tests which show that adjacent markers in *S. coelicolor* have cotransducible analogues in *S. venezuelae* (Stuttard, 1983*b*; C. Stuttard, S. Vats & L. Atkinson, unpublished data). Since most *Streptomyces* linkage maps of genes involved in primary metabolism are very similar (Hopwood *et al.*, 1983), it is of interest to note that the relative locations of genes involved in secondary metabolism, especially the biosynthesis of different antibiotics, are quite varied. Thus in *S. coelicolor* A3(2), genes for the synthesis of three antibiotics, actinorhodin, a calcium-dependent antibiotic and undecylprodigiosin, are located in three well-separated clusters (*act*, *cda* and *red* loci, respectively) (Hopwood *et al.*, 1985). In *S. rimosus* there are two clusters of oxytetracycline genes in diametrically opposite locations at the 3 o'clock and 9 o'clock positions (Rhodes *et al.*, 1981); and in *S. erythreus* the *ery* genes are at 7 to 8 o'clock (Weber *et al.*, 1985). The location of *cml* genes between *cys-28* and *pdxB* (S. Vats and C. Stuttard, unpublished transduction data) at 1–2 o'clock on the *S. venezuelae* map is close to a possible location of aromatic pathway (*aro*) genes (by analogy with *S. coelicolor*). Therefore, it will be of interest to determine whether *cml* and *aro* genes are adjacent in *S. venezuelae*, and whether this proximity is involved in the regulatory switch between the primary and secondary metabolism of chorismic acid in *S. venezuelae*.

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