The Circular Linkage Map of *Streptomyces rimosus*

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SUMMARY

A circular map of two strains of *Streptomyces rimosus* was constructed by the analysis of several hundred heteroclones. Segregants from four-point and five-point heteroclones were scored and the markers arranged by the criterion of minimizing the number of quadruple crossovers. Distances between genes were estimated from the analysis of the segregation of pairs of loci, whenever the ratio between the frequency of parental segregants approached the ratio between those of recombinant segregants. The nutritional markers appear to be asymmetrically distributed around the map, the upper arc containing a larger proportion of genes. The arrangement of genes in the upper arc recalls that of *S. coelicolor* A3(2), with which *S. rimosus* forms fertile crosses. The 'empty' regions known in the *S. coelicolor* map may be absent in *S. rimosus*.

INTRODUCTION

Data on the linkage map of *Streptomyces rimosus* have accumulated in recent years and show an increase in interest in the transfer of the experience gained with *S. coelicolor* (Hopwood, 1967) to a species of industrial concern. The first evidence of linkage was reported by Alačević (1969, 1973) and a preliminary circular arrangement of a dozen loci was presented by Vešligaj & Alačević (1972). A circular sequence of 24 genes was reported by Friend & Hopwood (1971) but information on the relative lengths of map intervals was limited by the method of analysis adopted, i.e. study of the segregation of unselected markers on media selecting for pairs of markers in repulsion. The data presented here were obtained using a completely different approach, i.e. analysis of heteroclones (Hopwood, Sermonti & Spada-Sermonti, 1963), which provides information on map distances, when particular conditions are fulfilled. Several hundred heteroclones have been analysed over a period of more than five years, and the results appear to be in substantial agreement with those reported by Friend & Hopwood (1971) and suggest some peculiarities in the map of *S. rimosus* when compared with the better known map of *S. coelicolor* (Hopwood, 1967).

METHODS

Strains and media. Two different strains of *Streptomyces rimosus* were employed: *S. rimosus* ATCC 10970^T (R7) and a *S. rimosus* soil isolate, from the culture collection of the Faculty of Technology, Zagreb (R6). Strain R7 has very low activity (100 to 200 µg/ml) and strain R6 yields about 2000 µg oxytetracycline/ml. The two strains differ in certain morpho-

logical characteristics: form of sporophores, colony shape and pigment production. Strain R7 grows well with abundant sporulation and sporophores of rectus-flexibilis type (RF) and forms a pale yellowish brown pigment. Strain R6 has compact dark brown colonies with spiral sporophores (S). Strain R7 grows as well at 28 °C as at 37 °C while strain R6 grows much better at 28 °C.

Most mutant strains were isolated after treatment of spores with u.v. light, and a few after exposure to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). Mutants obtained by MNNG and some mutants obtained by u.v. were kindly supplied by V. DeliĆ. The mutant strains employed in our experiments were double or triple auxotrophs. Before crossing, they were checked by auxanography and for back-mutation.

A complete medium (CM) for maintenance of stock cultures and for crosses was employed, with the following ingredients (g/1000 ml distilled water): Bacto-peptone, 2.00; yeast-extract, 4.00; MgSO₄·7H₂O, 0.50; hydrolysed casein (Difco), 2.00; glucose, 10.00; agar, 20.00.

A minimal medium (MM) for detection of auxotrophic mutants and characterization and isolation of recombinants contained the following ingredients (g/1000 ml distilled water): L-asparagine, 0.50; K₂HPO₄, 0.50; glucose, 10.00; agar, 20.00. All media were adjusted to pH 6.8 to 7.0 after sterilization.

Growth factors used to supplement MM were added at the final concentration reported by Hopwood & Sermonti (1962).

Crossing procedures. Crosses were carried out by the technique employed by Hopwood & Sermonti (1962). Crosses of type R7 × R7 and R7 × R6 were incubated for 5 days and those of type R6 × R6 for 5 to 7 days. Mixed cultures were made on agar slant on complete medium or on cellophane discs as described by Sermonti, Bandiera & Spada-Sermonti (1966).

Detection and analysis of heteroclones. Identification, isolation and analysis of heteroclones were carried out as described by Sermonti (1969) for *Streptomyces coelicolor*. In most cases the heteroclones were detected as minute colonies growing on cellophane discs seeded with several thousand spores of both parents, kept one day on CM and transferred to MM where they emerge. The microcolonies were finely broken by a needle in a drop of water and the suspension obtained was spread on to CM at a concentration to give separate colonies. These were transferred to master plates of CM, and then tested.

Analysis of auxotrophs. The nutritional requirement of segregants was usually scored by replica-plating the master plates to diagnostic media. When the result of replica-plating was not clear cut, as was often the case, the requirements were checked by means of auxanographic tests to rule out any possible misclassification.

When the analysis was limited to marker sequence, the data of heteroclones obtained from the same cross were usually pooled.

ANALYTICAL PROCEDURES

Arrangement of markers in four-point heteroclones. Only three circular permutations of four markers are possible. The criterion of choice between them was to adopt the one(s) fitting the observed segregants with the minimum requirement for quadruple crossing-over. Only segregants bearing two markers from either parent are relevant in this respect. Any such segregant conflicts with one possible permutation. The ideal situation is met when two (non-complementary) relevant segregants are present, to leave only one possible permutation unaffected by quadruple crossing over (Tables 2, 3). When only one permutation is excluded, some information is still available, the genes being classifiable in two non-overlapping pairs,

Table 1. List of mutant alleles in *Streptomyces rimosus* R7 and R6

Alleles*	Requirement	Corresponding locus in <i>S. coelicolor</i> A(3)2	Linkage
<i>ade-14</i>	Adenine	<i>adeA</i> or <i>B</i>	—
<i>ade-18,19,20</i>		<i>adeA</i> or <i>B</i>	Close to <i>ade-9</i> †
<i>ade-9</i> †		<i>adeA</i> or <i>B</i>	Close to <i>ade-18,19,20</i>
<i>arg-1</i>	Arginine	<i>argB</i>	Close to <i>arg-2,30,44,51</i> †
<i>arg-2</i>		<i>argB</i>	Close to <i>arg-1,30,44,51</i> †
<i>arg-30, 51</i> †	Arginine or ornithine or citrulline	<i>argA</i> or <i>C</i>	Close to <i>arg-1,2,44</i>
<i>arg-44</i>	Arginine	<i>argB</i>	Close to <i>arg-1,2,30,51</i> †
<i>arg-49</i>		<i>argB</i>	—
<i>cys-102,113</i> †	Cysteine, cystine or S ₂ O ₃	—	—
<i>gua-2</i>	Guanine	<i>guaA</i> or <i>B</i>	—
<i>his-1,6</i>	Histidine or histidinol (imi- dazole glycerol phosphate dehydrase)	<i>hisB</i>	Close to <i>his-2,24,4,21</i>
<i>his-2,24</i>	Histidine (histidinol dehydrogenase)	<i>hisA</i>	Close to <i>his-1,6,4,21</i>
<i>his-3,16,17</i>	Histidine or histidinol (his- tidinol phosphate phosphatase)	<i>hisD</i>	Distant from <i>his-1,6,2,24,4,9</i> †, <i>21</i>
<i>his-4,9</i> †, <i>21</i>	Histidine or histidinol	<i>hisC, I</i> or <i>F</i>	Close to <i>his-1,6,2,24</i>
<i>ilv-2</i>	Isoleucine and valine	<i>ilvA</i> or <i>B</i>	—
<i>leu-2</i>	Leucine	<i>leuA</i> or <i>B</i>	—
<i>lys-4</i>	Lysine	<i>lysA</i>	—
<i>met-95,108,117</i>	Methionine	<i>metA</i>	Close to <i>met-109,115</i>
<i>met-112</i> †		<i>metA</i>	—
<i>met-109,115</i>	Methionine or homocysteine	<i>metB</i>	Close to <i>met-95,108,117</i>
<i>nic-1,5,6</i>	Nicotinamide	<i>nicA</i> or <i>B</i>	Distant from <i>nic-3</i> †
<i>nic-3</i> †		<i>nicA</i> or <i>B</i>	Distant from <i>nic-1,5,6</i>
<i>pan-1,2</i> †, <i>9</i>	Pantothenic acid	—	—
<i>pro-1,11,12</i> †	Proline	<i>proA</i>	—
<i>pdx-9</i>	Pyridoxine	<i>pdxA</i>	—
<i>pdx-10</i>		<i>pdxA</i>	—
<i>rib-4,5</i>	Riboflavine	<i>ribA</i> or <i>B</i>	—
<i>try-3,12</i>	Tryptophane	<i>tryA</i>	Close of <i>try-5</i>
<i>try-5</i>	Tryptophane, indole or anthranilic acid	<i>tryB</i>	Close to <i>try-3,12</i>
<i>tyr-1,6,10,11</i>	Tyrosine	<i>tyrB</i>	—
<i>ity-2</i> †	Tyrosine and tryptophane	—	—
<i>ura-4</i>	Uracile	<i>uraB</i> or <i>C</i>	—

* Grouped numbers indicate allelic mutations.

† Mutants of *S. rimosus* R6 (Pigac, 1972).

e.g. (*ab*) (*cd*), which makes the permutations ...*abcd*... and ...*abdc*... possible, but rules out ...*acbd*....

Arrangement of markers in five-point heteroclones. The same general criterion of minimizing the number of crossovers was applied to five-point heteroclones. The situation is, however, complicated by the fact that there are twelve possible circular permutations of five markers and ten segregants which involve quadruple crossovers on any hypothesis. By means of the scheme in Table 4 the choice among the possible permutations is easily made. The order requiring the minimum total sum of quadruple crossovers was adopted. As a rule two, and sometimes more, alternatives are equally compatible (Table 5), and they often leave undecided the order of a pair of adjacent markers (in some cases two pairs). Five-point crosses utilize more information than the four-point ones, the number of possible relevant genotypes being

Table 2. *Method for arranging loci by four-point heteroclones*

	Segregants involving quadruple crossover*					
	cross: $\frac{a \quad b \quad + \quad +}{+ \quad + \quad c \quad d}$			cross: $\frac{a \quad b \quad c \quad +}{+ \quad + \quad + \quad d}$		
	Phenotypes					
Possible permutation	$\frac{+}{abcd}$	$\frac{ad}{bc}$	$\frac{bd}{ac}$	$\frac{c}{abd}$	$\frac{b}{acd}$	$\frac{a}{bcd}$
I... <i>a b c d</i> ...		●			●	
II... <i>a c b d</i> ...	●			●		
III... <i>a b d c</i> ...			●			●

* Each spot means that segregants with the given phenotype(s) (column) involved quadruple crossover on the assumption of the order of markers (row) indicated at left. Only 6 out of 16 genotypes are relevant. The spot under *ad* and *bc* (first row, second column) indicates, for instance, that either genotype involves quadruple crossover on the assumption of permutation I (coupling of markers as above).

Table 3. *Choice of gene arrangement involving the minimum number of quadruple crossovers in a four-point heteroclone**

Cross: <i>nic-1</i> <i>rib-4</i> <i>pdx-9</i> × <i>leu-2</i>										
Code: <i>a</i> <i>b</i> <i>c</i> <i>d</i>										
Segregants observed										
Relevant						Non-relevant				
Genotype		Code	No.	Genotype				No.		
+	<i>rib</i>	+	+	<i>b</i>	21	<i>nic</i>	<i>rib</i>	<i>pdx</i>	+	27
+	+	<i>pdx</i>	+	<i>c</i>	30	<i>nic</i>	<i>rib</i>	+	+	1
						<i>nic</i>	+	<i>pdx</i>	+	2
						+	<i>rib</i>	<i>pdx</i>	+	9

No. of segregants involving quadruple crossover

Possible gene permutations	Genotype (code)			Totals
	<i>c</i>	<i>b</i>	<i>a</i>	
I <i>a b c d</i>	—	21	—	21
II <i>a c b d</i>	30	—	—	30
III <i>a b d c</i>	—	—	0	0

Gene permutation involving minimum crossover

* See Table 2, right half.

Table 4. Method for arranging loci in five-point heteroclones

		Segregants involving quadruple crossover*									
		+	a	b	c	ad	ae	bd	be	cd	ce
		abcde	bcde	acde	abde	bce	bcd	ace	acd	abe	abd
I	... a b c d e ...			●			●	●	●	●	
II	... a b e d c ...		●			●	●		●	●	
III	... a c b d e ...				●		●	●		●	●
IV	... a e b c d ...	●		●	●				●	●	
V	... a e b d c ...	●	●		●		●			●	
VI	... a e c b d ...	●		●	●			●		●	●
VII	... a b e c d ...	●	●	●		●			●		
VIII	... a b d c e ...	●		●			●	●			
IX	... a c e b d ...	●	●		●	●		●			●
X	... a b c e d ...			●		●		●	●		●
XI	... a b d e c ...		●			●	●	●			●
XII	... a c b e d ...				●	●			●	●	●

* Each spot means that segregants with the given phenotype(s) (column) involved quadruple crossover on the assumption of the order of genes (row) indicated at the left. Most phenotypes are irrelevant.

The segregants' markers are to be referred to the symbols *a*, *b*, *c*, *d* and *e*. Each spot is replaced by the number corresponding to the frequency of the phenotype(s) indicated above and the values on each of the twelve rows are summed up: the lowest value(s) correspond(s) to the permutation(s) involving the minimum numbers of crossovers to give the observed segregants.

twenty compared with six. They provide in turn more information, the possible permutations being twelve instead of three.

Location of new markers by the help of allele frequency gradients. The frequencies of the alleles of either parent were calculated in each heteroclone. When the markers had been arranged by the above mentioned criteria, the frequency gradient usually appeared continuous, and it sometimes helped in choosing between two (or more) arrangements which both minimized the number of crossovers. In some cases, however, it showed discontinuities which were possible due to multiple deletions in the zygote structure or to differential viabilities. This makes estimation of the gradient an unreliable method, to be used only subsidiarily. It was of some value when a new marker was to be located among others of known order. In one heteroclone, for instance, the following allele gradient was observed (the figures refer to the allele frequencies of one parent):

$$\frac{his-4 \quad pdx-9^+ \quad rib-4^+ \quad nic-1^+}{19 \quad 87 \quad 127 \quad 153}.$$

An allele, *gua-2*, of unknown location, had a frequency of 58. This suggested its location between *his-4* and *pdx-9*⁺, which is its location as deduced by other observations.

Analysis of trios of loci. The information obtainable by analysis of segregation of trios of loci was generally unsatisfactory in *Streptomyces rimosus*. When three linked markers are in a linear order, one pair of recombinant complementary classes should appear with a very low frequency, the double crossover classes. This was rarely the case, all the three pairs of recombinant classes usually appeared at similar frequencies. This was possibly due to the relative shortness of the 'disomic' region in the heteroclones examined. Although in some cases analysis of segregation of trios of loci was of some help in clarifying dubious cases, it will not be referred to in this paper.

Table 5. *Choice of gene arrangement involving the minimum number of quadruple crossovers in a five-point heteroclone*

Cross: <i>nic-1</i> <i>pan-1</i> <i>rib-4</i> × <i>try-1</i> <i>his-1</i>												
Code: <i>a</i> <i>b</i> <i>c</i> <i>d</i> <i>e</i>												
Segregants observed												
Relevant						Non-relevant						
Genotype					Code	No.	Genotype					No.
+	+	+	+	+	+	15	<i>nic</i>	<i>pan</i>	<i>rib</i>	+	+	13
<i>nic</i>	+	+	+	+	<i>a</i>	10	+	+	+	<i>try</i>	<i>his</i>	11
+	+	<i>rib</i>	+	+	<i>c</i>	21	<i>nic</i>	+	<i>rib</i>	+	+	156
							<i>nic</i>	+	+	<i>try</i>	+	10
No. of segregants involving quadruple crossover												
Genotype (code)												
Possible gene permutations					+	<i>a</i>	<i>c</i>	Others			Totals	
I	<i>a b c d e</i>				—	—	—	0			0	
II	<i>a b e d c</i>				—	10	—	0			10	
III	<i>a c b d e</i>				—	—	21	0			21	
IV	<i>a e b c d</i>				15	—	21	0			36	
V	<i>a e b d c</i>				15	10	21	0			46	
VI	<i>a e c b d</i>				15	—	21	0			36	
VII	<i>a b e c d</i>				15	10	—	0			25	
VIII	<i>a b d c e</i>				15	10	—	0			25	
IX	<i>a c e b d</i>				15	10	21	0			46	
X	<i>a b c e d</i>				—	—	—	0			0	
XI	<i>a b d e c</i>				—	10	—	0			10	
XII	<i>a c b e d</i>				—	—	21	0			21	
Gene permutations involving minimum crossover:												
I	<i>a b c d e</i>	} i.e. <i>a b c (d e)</i> , corresponding to:			<i>nic-1 pan-1 rib-4 (try-1 his-1)</i>							
X	<i>a b c e d</i>											

Recombination between pairs of loci. As discussed by Hopwood & Sermoniti (1962) for *Streptomyces coelicolor*, the recombination frequencies between pairs of loci are reliable in heteroclones only when a single disturbing factor affects the parental and the recombinant classes, i.e. when the ratio between parental segregants does not significantly differ from the ratio between recombinant segregants. This was rarely the case in *S. rimosus*; nevertheless a series of reliable data have been collected, which are consistent and give 'map distances' showing reasonable additivity.

RESULTS

Ordering of loci in four- and five-point heteroclones. The sequence of markers in seventy heteroclones from twenty-five crosses is illustrated in Table 6. The segregations observed were analysed by the methods summarized in Tables 2 and 4. Heteroclones from only 15 crosses permit an unequivocal assignment of the genes, the others leaving two or sometimes four alternatives undecided. The reliability of the assignment of the loci is confirmed by the number of quadruple crossovers involved by the chosen sequence when compared with the higher numbers involved by the discarded ones (last two columns in Table 6). This kind of elaboration takes into account only a small proportion of the information provided by the heteroclones; about one-fifth (6/32) of the possible segregants in four-point hetero-

Table 6. Sequence of markers in some heteroclones

No. hetero-clones	Total no. segregants examined	Sequence of markers	No. quadruples involved	
			by the given sequence(s)	by other sequence(s)
2	207	<i>pro-1 his-4 gua-2 tyr-1</i>	2	10, 41
1	82	<i>pro-1 ilv-1 his-4 tyr-1</i>	0	4, 12
2	337	<i>pro-1 his-4 tyr-1 leu-2</i>	8	28, 56
3	114	<i>his-1 arg-1 tyr-1 pro-1</i>	0	3, 23
2	184	<i>pdx-9 leu-2 rib-4 nic-1</i>	0	29, 41
3	175	<i>his-24 pdx-9 rib-4 nic-1</i>	2	5, 7
3	191	<i>(met-95 arg-1) (pan-1 pro-1)</i>	0, 0	66
3	284	<i>(his-6 arg-1) (rib-5 nic-1)</i>	0, 0	9
2	141	<i>(his-4 arg-1) (rib-5 nic-1)</i>	0, 0	54
2	199	<i>(nic-1 arg-1) (rib-4 pan-1)</i>	0, 0	32
3	217	<i>(his-4 arg-30) (rib-4 nic-1)</i>	2, 3	30
3	395	<i>(nic-1 met-95) (rib-5 nic-1)</i>	0, 1	165
5	1037	<i>(his-4 gua-2) (arg-1 pan-1)</i>	0, 0	450
2	203	<i>(met-95 his-4) (try-3 pan-1)</i>	0, 3	91
2	202	<i>(nic-1 met-95) (rib-4 pan-1)</i>	0, 1	88
2	420	<i>(his-4 try-5) (arg-30 tyr-1)</i>	5, 8	21
4	738	<i>(ura-4 lys-4) (his-4 gua-2)</i>	0, 0	8
2	366	<i>ura-4 lys-4 ade-19 pdx-9 rib-4</i>	0	5 ... 34
2	564	<i>pan-1 nic-1 (his-4 try-3) rib-4</i>	0, 0	33 ... 212
4	700	<i>pan-1 nic-1 (his-4 tyr-1) rib-4</i>	6, 8	76 ... 192
2	365	<i>pan-1 nic-1 (his-1 tyr-1) rib-4</i>	10, 11	26 ... 74
3	232	<i>(pan-1 nic-1) (pro-1 arg-1) rib-4</i>	0, 0, 3, 3	11 ... 123
2	164	<i>(nic-1 met-108) his-4 (try-3 pan-1)</i>	0, 0, 1, 1	33 ... 88
2	181	<i>(rib-4 nic-1) (his-4 gua-2) pdx-9</i>	4, 4, 4, 4	13 ... 49
5	478	<i>his-4 (gua-2 ade-19) (pdx-9 rib-4)</i>	3, 3, 4, 4	14 ... 157

Order of markers in *Streptomyces rimosus* as deduced from the data reported above.

clones and about one-third (20/64) in five-point ones. In this respect selective analysis (Friend & Hopwood, 1971) is more convenient. The irrelevant segregants are considered in the analysis of allele gradients, as well as of trios or pairs of loci, i.e. in those analytical procedures which provide information on distances between loci, and require the consideration of all observed crossovers.

The data in Table 6 fit the order of the markers shown in the footnote to it. A dozen markers are arranged in unequivocal order, while a few are left in alternative locations. This order is also based on allelism or close linkage between mutations of similar phenotype (e.g. *his-1,4,6,24*), which have been discussed by Alačević (1969, 1973) and Vešligaj & Alačević (1972).

Gradient of markers in heteroclones. Due to the variable structure of the heteroclones, allele frequencies do not provide direct information on the relative positions of the loci. In most heteroclones the frequencies of the alleles derived from one parent appear in a continuous gradient corresponding to their sequence. This is the case for heteroclones with a single

Table 7. *Example of the use of allele frequencies in ordering of markers from five heteroclones**

<i>his-4</i>	<i>gua-4</i>	<i>ade-19</i>	<i>pdx-9</i>	<i>rib-4</i>	<i>nic-1</i>	Totals	No. quadruple cross-overs required
73	63	—	52	36	15	83	2
72	69	—	58	34	8	98	2
99	56	39	3	2	—	148	2
58	32	22	0	0	—	94	0
51	43	32	2	1	—	97	0

* The frequencies of the alleles contributed by one parent are given.

Table 8. *Some distances between pairs of loci, in a clockwise sense*

Pairs of alleles		Segregant's number*				Recombinants (%)	Approx. average†	
		Parental		Recombinant				
<i>pro-I</i>	<i>his-4</i>	105	22	14	4	9	10	
		144	19	20	3	12		
		107	19	10	4	10		
		128	27	5	1	4		
	<i>arg-I</i>	42	5	8	2	16	20	
		39	39	9	2	13		
		89	43	39	18	30		
	<i>tyr-I</i>	74	55	10	6	11	(25)	
		40	9	9	3	20		
		25	9	14	2	32		
		45	2	28	7	42		
	<i>pdx-9</i>	40	5	34	1	43	(45)	
	<i>his-I,4</i>	<i>arg-30</i>	112	54	5	2	4	5
			85	36	5	3	6	
	<i>tyr-I</i>	50	40	4	4	8	15	
		64	2	21	1	25		
		26	13	9	5	26		
		105	68	14	5	10		
		106	21	11	2	9		
		<i>ade-19</i>	121	3	26	0		17
<i>pdx-9</i>	38	11	28	3	39	(35)		
	46	2	49	0	50			
	35	31	5	3	11			
<i>arg-I,30,44</i>	<i>tyr-I</i>	108	43	18	2	12	10	
		22	18	5	1	13		
	<i>pdx-9</i>	81	4	33	1	28	(30)	
<i>tyr-I</i>	<i>ade-19</i>	82	2	16	0	16	(15)	
<i>ade-19</i>	<i>pdx-9</i>	59	40	9	7	13	15	
		115	37	35	6	21		
	<i>rib-4</i>	137	23	15	1	9	20	
		111	20	14	1	10		
		57	38	11	9	18		
		114	36	36	7	27		

Table 8. (cont.)

Pairs of alleles		Segregant's number*				Recombinants (%)	Approx. average†
		Parental		Recombinant			
<i>pdx-9</i>	<i>rib-4</i>	46	32	12	9	21	15
		51	14	12	2	17	
		95	4	19	1	17	
		118	93	13	12	10	
	<i>nic-1</i>	28	26	13	13	30	40
		39	32	14	13	27	
		62	8	89	11	59	
		62	8	64	3	49	
<i>rib-4,5</i>	<i>pan-1</i>	56	19	18	6	24	20
		110	16	27	4	19	
	<i>nic-1</i>	35	25	29	25	48	40
		104	33	65	15	40	
44		12	24	3	32		
39		5	24	2	37		
	<i>pan-1</i>	28	12	22	11	45	25
		36	16	30	15	46	
		107	5	63	4	37	
		47	19	12	7	22	
<i>pan-1</i>	<i>nic-1</i>	58	2	38	1	29	25
		74	3	22	0	22	
		60	3	37	1	38	
		<i>his-4</i>	111	5	36	6	27
100			1	92	0	47	
93			55	27	5	17	(15)
<i>nic-1</i>		<i>pro-1</i>	74	8	16	1	17
	<i>his-4</i>	64	3	32	1	33	25
	<i>arg-1</i>	91	33	17	5	15	
		71	2	18	0	30	
	<i>tyr-1</i>	160	2	192	0	54	(50)

* Each line refers to a single heteroclone.

† The averages are rounded to the nearest five units, the least reliable estimates are in brackets.

disomic region (Hopwood, 1966), and is the ideal situation for locating a new marker within a series of known ones. In other cases, however, the sequence of markers forms a double gradient around a maximum central value, as expected from heteroclones with two disomic regions (Sermonti, 1969). One such example is a heteroclone, in which the ordered series of alleles (contributed from one parent) *nic-1*, *his-4*, *try-5*, *rib-4*, *pan-1* show the following frequencies: 42, 64, 100, 42, 2 (132 segregants). In such cases an unknown marker with a given frequency may be located in two positions, and the choice must rely on the minimum-crossover criterion to discriminate between the two alternative hypotheses. Unfortunately rare cases are also met in which several irregularities are present, possibly due to viability effects.

Estimation of map intervals. The recombination frequency was estimated for many pairs of loci, with satisfactory agreement. The data in Table 8 and summarized in Fig. 1 refer to nine key loci, most frequently involved in crosses. The variability of the data is such that they have been finally rounded off to five units of recombination and no statistical analysis has been attempted. The further one marker appears from another in the sequence deduced from the criteria discussed in the preceding paragraphs, the larger the distance in map units

Fig. 1. Approximate distances, in recombination units, for nine key markers in *Streptomyces rimosus*. The values are those reported in Table 8.

appears. Furthermore reasonable additivity of distances was observed, supporting the reliability of the estimated map intervals. Recombination frequencies approaching 50 % were consistently observed for markers located at opposite sides of the map. The distance between pairs of consecutive loci never exceeded 25 units (*pan-1* to *nic-1*), a fact which supports the circularity of the map as well as the absence of large 'empty' regions like those observed in *Streptomyces coelicolor* (Hopwood, 1967). From the map intervals calculated (Table 8), a preliminary map showing distances between loci was built up, amounting to a total length of about 130 to 150 units.

Localization of other genes on the map. Some genes have been mapped with reference to the key markers reported in Fig. 1, on the basis of a variety of experiments (Fig. 2). Mutations *pdx-10*, *tty-2* and *cys-112* have been located, between *pan-1* and *pro-1*, by selective analysis of many crosses carried out with the producer strain (Pigac, 1972). Allele *met-95* was placed between *pro-1* and *his-1* or *his-4* by the analysis of many heteroclones and according to the estimate of its distance from *pro-1* and *arg-1*. Similarly *ilv-2* was placed between *pdx-9* and *ade-19*, to both of which it appears very closely linked, and is more distant from *rib-4* or *rib-5*. Markers *ura-4* and *lys-4* are linked to each other and are close to the group of mutations *his-1*, *gua-2*, *try-1* and *ade-19*. From the evidence of heteroclone segregation *leu-1*

Fig. 2. The map of *Streptomyces rimosus*. The loci marked at intervals are those shown in Fig. 1. Certain loci have not been ordered relative to loci covered by broken lines. The other loci are spaced according to their approximate distance; the whole map represents about 140 recombination units.

appeared to be in the region between *pdx-9* and *rib-4* (Table 3). Finally *his-16* and *his-17* (allelic), corresponding to the gene *his-D* of *Streptomyces coelicolor*, turn out to be far from the main cluster of *his* genes, but their position has not yet been reliably established. If the map is oriented to give the best overlap with that of *S. coelicolor* (see Discussion) most of the genes appear to be located in the upper arc of the map. This is very likely the result of an unequal distribution of nutritional genes, although it may be partly due to the larger number of reference markers in that region. Some of the genes in undeterminate positions might belong to the lower arc.

DISCUSSION

The map of *Streptomyces rimosus* ATCC10970 fits a circular model, since no discontinuity in the linkage of markers was observed. Moreover, although several hundred heteroclones have been tested, no single case was found in which a pair of markers segregated indepen-

dently from another. In contrast, this was so frequent in *S. coelicolor* that a map with two linkage groups was first postulated (Hopwood & Sermonti, 1962). Later the two linkage groups were shown to be continuous from evidence that, when two unselected loci were not separated by selected markers, they never segregated independently (Hopwood, 1969). The same evidence was provided by Friend & Hopwood (1971) for a strain of *S. rimosus*. Although largely comparable with that of *S. coelicolor* the map of *S. rimosus* (Friend & Hopwood, 1971) shows some singularities revealed by the analysis of heteroclones.

The general lack of independence in the segregation of any two pairs of loci around the map indicates that the auxotrophic markers are not clustered in two groups spaced by two 'empty' regions as in *S. coelicolor*. This possibility was also mentioned by Friend & Hopwood (1971).

Another interesting feature of the map of *Streptomyces rimosus* ATCC 10970 is the non-random distribution of auxotrophic mutations. The upper arc of the map, from 9 to 3 o'clock, contains more markers than the lower arc. This pattern does not emerge from the data of Friend & Hopwood (1971). The same pattern, however, is also apparent in the map of *S. coelicolor* presented by Hopwood (1967). Against 42 nutritional genes in the upper arc, only 25 such genes are found in the lower arc ($\chi^2 = 3.985$; $P < 0.05$).

The conclusion derived from the analysis of heteroclones may be biased by the occasional recovery of heteroclones derived from several acts of conjugation. This situation would not, however, affect the ordering of loci, which is usually worked out from data obtained by pooling segregants collected from a series of heteroclones. It could, however, affect estimates of map distance, but only in the sense of providing larger estimates for short distances. This would not affect the main conclusions of this work, which involve the observation of the shortening of some intervals, in reference to those reported in *Streptomyces coelicolor* A3(2) (Hopwood, 1967).

Due to the scarcity of markers in the lower arc, comparison can be made only between the upper arc of *Streptomyces coelicolor* A3(2) and the upper arc of *S. rimosus* ATCC 10970 (R7). The orientation of the map of *S. rimosus* is based on the observed correspondence between the loci of the two species.

The sequence of the well-located markers *nic-1*, *pro-1*, *his-1,4,21,6,2,24*, *arg-1* and *try-1* in *Streptomyces rimosus* R7 corresponds not only in order but also in map intervals to the sequence *nicA*, *proA*, *hisB, I, A, (I, C, F)*, *argA, B*, and *aroA* in *S. coelicolor* A3(2). The markers *cys-112*, *met-95*, *try-3*, *gua-2*, *lys-4*, *ura-4* and *leu-2*, not yet unequivocally located, probably correspond in position to the following loci in *S. coelicolor* A3(2): *cysA*, *metA*, *tryA, B* or *C*, *guaA*, *lysA*, *uraC, D* and *leuB*.

The map of *Streptomyces rimosus* R7 and R6 by no means conflicts with the map presented by Friend & Hopwood (1971), in so far as both are comparable to the map of *S. coelicolor* A3(2). *Streptomyces rimosus* R7 gives recombinants and heteroclones when crossed with a UF strain of *S. coelicolor* A3(2), although at a low rate. *Streptomyces coelicolor* appears to act as a recipient and crossing over occurs at normal frequency in various regions of the map in the heteroclones formed (Alačević, 1973). The sex factor of *S. rimosus* is transferable to *S. coelicolor* UF, converting it to IF (Alačević, unpublished data).

Streptomyces rimosus ATCC 10970 can hardly be defined as a different species to *S. coelicolor* A3(2) by genetic criteria, although they are clearly distinguishable in morphological traits, in the pigment formed, in antibiotic production, in resistance to streptomycin and in the optimal composition of the minimal medium. The most striking difference between the two organisms so far appears to be the occurrence of two 'empty' regions in the genome of *S. coelicolor* (Hopwood, 1967), which are not found in *S. rimosus*.

The comparative physiology and topology of genes involved in secondary metabolism and their possible transfer from one species to another by 'interspecific' crosses are among the most fascinating prospects for the genetics of *Streptomyces*.

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