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Construction and Properties of Hybrids Obtained in Interspecific Crosses between *Streptomyces coelicolor* A3(2) and *Streptomyces griseus* Kr.15

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SUMMARY

Recombinants between *Streptomyces coelicolor* A3(2) and *Streptomyces griseus* Kr.15 were obtained using methods of hybrid construction. Recombinant Rcg1, obtained from a cross between *S. griseus* and a *S. coelicolor* UF (SCP1$^-$) strain, phenotypically resembled *S. coelicolor* UF strains and in crosses with a *S. coelicolor* NF donor strain produced recombinant progeny at a frequency of 100 %. Recombinant Rcg3, like SCP1-carrying *S. coelicolor* strains, inhibited SCP1$^-$ strains of *S. coelicolor* and in crosses with a UF recipient strain of *S. coelicolor* generated recombinants at high frequency. In crosses between *S. griseus* and Rcg1 the frequency of recombinant formation was increased about 100-fold relative to crosses between *S. griseus* and *S. coelicolor*. Effective transfer of *S. griseus* and Rcg3 chromosomal markers into Rcg1 and *S. coelicolor*, respectively, indicated that *S. griseus* had donor properties.

Studies of the ability of recombinants to support phage growth indicated that parental chromosomal fragments containing genes involved in control of phage-receptor formation and intracellular growth were present in the hybrids. Grisin-producing recombinants, capable of restricting phages attacking *S. coelicolor* and *S. griseus*, were obtained.

INTRODUCTION

Cross-breeding procedures are used extensively in animal and plant breeding but have not been applied on a large scale for the selection of micro-organisms producing biologically active substances. The exploitation of techniques for obtaining hybrids of antibiotic-producing actinomycetes, especially hybrids between strains submitted to divergent selection and those between different actinomycete species, should facilitate the development of improved strains and enhance the possibility of their genetic analysis.

Studies of the capacity of various strains, species and genera of Enterobacteriaceae to form hybrids have shown that integration of a donor fragment depends on the extent of genetic and physical homology between the parental genomes (Demerec, 1965; Sanderson, 1971; Brenner & Falkow, 1971). Extensive studies of bacterial hybrids show that they may be helpful not only in solving taxonomic problems and in studying the behaviour of foreign genes in a different host, but also in elucidating peculiarities of host–phage relationships. For example, it has been possible to study the influence of the Shigella genome on the functioning of λ genes by constructing *Escherichia coli*–Shigella hybrids that have inherited *E. coli* genes controlling receptor synthesis for λ adsorption (Gemski, Alexeichik & Baron, 1972). It has also been shown that substitution of *E. coli* chromosomal genes by Klebsiella

nitrogen-fixation genes produces hybrids which, unlike the *E. coli* parental strain, cannot adsorb phage ϕX174 (Cannon *et al.*, 1974). Baron *et al.* (1970) and Friedman & Baron (1974) demonstrated that *E. coli–Salmonella typhosa* hybrids had acquired the ability of *E. coli* to adsorb phage λ, but the phage did not grow in these hybrids because they did not contain the bacterial locus involved in phage gene N expression. Using hybrids of *E. coli* Hfr and *Salmonella typhimurium* carrying various host specificity genes of *E. coli*, Colson & Colson (1972) identified a formerly undetected system of restriction and modification in Salmonella.

Effective exchange of chromosomal markers between derivatives of *Streptomyces coelicolor* A3(2) is known to take place via a process similar to conjugation in other bacteria and is promoted by the plasmid SCP1 (Vivian & Hopwood, 1970; Vivian, 1971; Hopwood *et al.*, 1973).

The similarity of the genetic maps of some representatives of the genus *Streptomyces* (Hopwood, 1967; Friend & Hopwood, 1971; Coats & Roeser, 1971; Alačević, Strašek-Vešligaj & Sermonti, 1973; Baumann & Kocher, 1976; Matselyukh, 1976) and the data on 'infectious' transfer of the plasmid SCP1 between various Streptomyces species (Hopwood & Wright, 1973) suggested that a study of hybrid formation within Streptomyces strains and species was feasible.

In a previous paper we reported crosses between *S. coelicolor* A3(2) and *Streptomyces lividans* 66, and described characteristics of the recombinants obtained (Alikhanian, Lomovskaya & Danilenko, 1976). In this paper we describe the methods used to construct hybrids between *S. coelicolor* A3(2) and *Streptomyces griseus* Kr.15, and the properties of these hybrids. Special attention is paid to the reaction of the hybrids to actinophages which are known to be capable of interfering with the industrial production of the antibiotic grisin. Grisin production is usually less in strains that are resistant to actinophages. Moreover, it is not easy to obtain, by selection, mutants which block the intracellular growth of virulent phages, or have lost the ability to adsorb a temperate phage. It is, therefore, advantageous to obtain hybrids which have received resistance genes from parental strains that are intrinsically phage resistant.

METHODS

Strains and phages. The actinomycete strains used are listed in Table 1. Genetic symbols are as given by Hopwood *et al.* (1973). Polyauxotrophic derivatives of *S. coelicolor* A3(2), strains 853, A585, A617, and actinophage VP5 of *S. coelicolor* were kindly supplied by D. A. Hopwood. Strains S18, S32, S179 and S187 were constructed as recombinants in crosses between derivatives of strain A3(2). *Streptomyces griseus* produces the antibiotic grisin (grisemin) (Korzybski, Kowszyk-Gindifer & Kuryłowicz, 1969; Krasilnikov, 1970). A derivative, K806, which produced more antibiotic than wild-type strains of *S. griseus* Kr.15, was selected after a single treatment of *S. griseus* Kr.15 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) as described by Delić, Hopwood & Friend (1970). The strain *S. griseus* K155 was obtained after a single treatment with ethylenimine (Alikhanian, 1969). Strain K155 required histidine and was resistant to phage Pg81. Phages Pg81, Pg100 and Pg56, acting on *S. griseus* Kr.15, and phages VP5 and ϕ448, acting on *S. coelicolor*, were used in host-range studies.

Media and techniques. Complete (CM) and minimal (MM) media and crossing procedures were essentially as described previously (Lomovskaya, Emeljanova & Alikhanian, 1971). Crosses between *str-s* and *str-r* strains were performed in two ways. (1) Spore suspensions of the parent strains were incubated separately for 6 h in peptone broth (Lomovskaya *et al.*,

Table 1. *Characteristics of actinomycetes used*

Strain			Genetic markers*
S. coelicolor A3(2)	wild type		IF prototroph
	A585		UF *adeCv10 mthB2 cysD18*
	A617		UF *pabA1 argA1 uraA1 strA1*
	853		NF *hisA1 adeA3 cysD18 uraA1*
	S186		UF *pheA1 strA1*
	S18		UF *adeCv10 mthB2 uraA1 cysD18 strA1*
	S32		UF *pheA1 strA1*
	S179		NF *hisA1 uraA1 pheA1*
	S187		NF *pheA1 hisA1*
S. lividans 66			UF prototroph
S. griseus Kr.15	K806		prototroph
	K155		*his-1*
Recombinants			
S. coelicolor A3(2)	Rcg1	(A617 × K806)†	*argA1 strA1*
× *S. griseus* Kr.15	Rcg2	(Rcg1 × K155)	*strA1*
	Rcg3	(A617 × K806)	*argA1 strA1*
	Rcg5	(Rcg1 × K155)	*argA1 strA1 his-1*
	Rcg7	(Rcg1 × K155)	*strA1*
	Rcg11	(S18 × K806)	*strA1*

* NF-like strains harbour the plasmid SCP1 integrated into the chromosome. UF strains lack the plasmid. IF strains contain the plasmid in the autonomous state (Vivian, 1971).

† Parental strains of an each recombinant are given in parentheses.

1972) on the surface of CM plates, and then germinated spores of each of the parents were sedimented and spread together on CM slants containing streptomycin (50 μg ml^{-1}). (2) Spore suspensions of the parent strains were mixed and grown on CM plates, which contained no streptomycin, for about 10 h: during this time, the parental strains did not produce sufficient antibiotic to arrest each other's growth. The mixed culture was then sedimented and transferred to CM slants containing streptomycin, to eliminate the *str-s* parental strain.

The progeny from single colonies formed on MM, which failed to give growth on the same medium and were probably heteroclones or heterokaryons, were analysed as follows. Suspended spores filtered through cotton wool were seeded on CM and the resulting single colonies were transferred to master plates and replica-plated to diagnostic media to analyse the colony genotypes.

The inhibiting properties of cultures producing antibiotics and the ability of SCP1-carrying strains to inhibit strains lacking the plasmid were tested by overlaying 24-h-old cultures on plates with spore suspensions of test cultures in soft agar.

The fertility of the progeny from crosses Rcg3 × A585 and S179 × Rcg1 was tested as described by Hopwood *et al.* (1969) and Vivian & Hopwood (1970). *Streptomyces coelicolor* A617, A585 and S32 were used as tester strains. Crosses of UF strains with the A3(2) IF strain served as controls. The UF tester strain chosen depended on the markers to be tested. Those of tested strains were situated in the fragment most often included in recombinants from the donor chromosome.

Phage techniques and antibiotic production tests were as described by Lomovskaya *et al.* (1971) and Zvenigorodsky *et al.* (1975). Adsorption of actinophages on to 6-h-old germinated spores was studied by adding phage to an equal volume of spore suspension at a low multiplicity of infection. The efficiency of adsorption was determined by measuring the decrease in free phages after centrifuging the mixture.

Table 2. *Generation of recombinants from crosses of S. coelicolor* A3(2) *strains in the presence and absence of streptomycin*

Cross	Selected markers	Recombination frequency on media: Without streptomycin	Recombination frequency on media: With added streptomycin
S187 NF × A617 UF	*uraA1*$^+$ *strA1*	6×10^{-1}	$3{\cdot}5 \times 10^{-2}$
853 NF × A617 UF	*argA1*$^+$ *strA1*	2×10^{-2}	$1{\cdot}5 \times 10^{-3}$
S187 NF × S18 UF	*cysD18*$^+$ *strA1*	5×10^{-3}	5×10^{-4}
A617 UF × A585 UF	*uraA1*$^+$ *strA1*	1×10^{-5}	$< 1 \times 10^{-8}$
	argA1$^+$ *strA1*	1×10^{-4}	$< 1 \times 10^{-8}$
	argA1$^+$ *adeC*$^+$	1×10^{-4}	$< 1 \times 10^{-8}$

RESULTS

Recombination between S. coelicolor A3(2) *and S. griseus* KT.15 *strains*

Mating in actinomycete crosses is known to occur in mixed cultures of *S. coelicolor* A3(2) and *S. griseus* KT.15 on CM. Since these two strains are mutually antagonistic, arresting each other's growth in mixed culture, it was impossible to grow them together and to ascribe *S. griseus* to any known fertility type. Thus, it was necessary to create conditions which would allow normal crossing between the strains. It would be possible, if crosses were made in the presence of streptomycin, to eliminate one of the parent strains during the early stages of mating (Hopwood, 1973). In a model cross between *str-s* NF (donor) and *str-r* UF (recipient) strains in which spores of the parents were germinated separately and then mixed on medium containing streptomycin, recombinants emerged at low frequency (Table 2). This indicated that, as in crosses between Hfr *str-s* and F$^-$(*str-r*) strains of *E. coli* (Hayes, 1952), an actinomycete *str-s* donor can donate its chromosome or chromosomal fragment to a *str-r* recipient in the presence of streptomycin. Recombinants should arise under these conditions provided that a conjugational type of transfer and not heterokaryosis takes place. No recombinants were detected in crosses between *str-s* and *str-r* UF strains in which spores germinated separately without streptomycin were mixed and crossed in the presence of streptomycin. Lack of recombinants in this case might indicate that recombinants from the UF × UF cross were formed due to genetic exchange in heterokaryons (Table 2).

Because the fertility type of *S. griseus* KT.15 was not known, crosses were conducted using two combinations: (i) using a *str-r* recipient UF strain of *S. coelicolor* and a *str-s* strain of *S. griseus*; and (ii) using a *str-s* NF donor strain of *S. coelicolor* and a *str-r* strain of *S. griseus*. In crosses of the first type (K806 × A617) in the presence of streptomycin, conducted in the same way as in the model cross, we detected colonies growing on selective plates (selected markers *uraA1*$^+$ *strA1*). Colonies that developed only substrate mycelium appeared at a frequency of approximately 10^{-4}. After incubation for 10 to 12 days, some colonies formed patches with aerial mycelium and spores (Fig. 1). Parental strains did not develop colonies of this type in the same conditions. On transfer to the same selective plates, the colonies failed to grow. Spores from each of 30 colonies were seeded on CM plates. In the progeny of 28 colonies only clones with the genotype of the parental strain *S. coelicolor* A617 were detected. Analysis of the progeny from the other two colonies showed that, from the first colony, 184 out of 200 clones analysed had the genotype of strain A617 and 16 were *argA1 strA1*, and, from the second colony, 191 of 200 clones analysed had the genotype of strain A617 and nine were *argA1 strA1*.

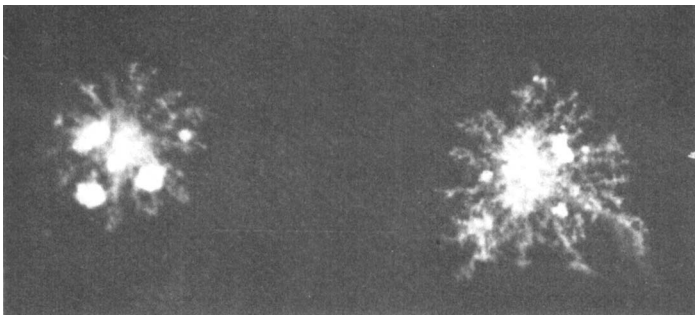

Fig. 1. Colonies arising on selective medium from crosses between *S. griseus* K806 and *S. coelicolor* A617 (selected markers *uraA1*$^+$ *strA1*). Patches of aerial mycelium and spores are shown.

Characteristics of recombinants from crosses between S. griseus K806 *and S. coelicolor* A617

ArgA1 strA1 recombinants failed to produce the antibiotic that arrested the growth of both SCP1-carrying and SCP1$^-$ *S. coelicolor* strains. Recombinants from the progeny of the first colony, in contrast to those from the progeny of the second colony, had the inhibiting properties characteristic of *S. coelicolor* strains carrying the plasmid SCP1. A representative of similar recombinants from the progeny of the first colony was designated Rcg3, and one from the progeny of the second colony was designated Rcg1. *Ura* clones were not detected among 10^4 progeny of both recombinants, suggesting that these recombinants showed no segregation in respect of *ura*$^+$ donated with the fragments of the *S. griseus* chromosome. Rcg3 inhibited an SCP1$^-$ *S. coelicolor* strain, but did not affect an SCP1-carrying strain and was resistant to the antibiotic produced by a strain carrying the SCP1 plasmid. These results indicated that, though derived from a cross of *S. griseus* with a *S. coelicolor* UF (SCP1$^-$) strain, Rcg3 phenotypically resembled strains bearing the SCP1 plasmid. In contrast, Rcg1 failed to arrest the growth of SCP1$^-$ strains and was sensitive to the antibiotic produced by an SCP1-carrying strain. Both recombinants were inhibited by *S. griseus* (Table 3, Fig. 2).

We examined the behaviour of Rcg3 in crosses with a *S. coelicolor* UF strain. In a cross with strain A585, recombinants arose at high frequency, as in crosses between *S. coelicolor* NF and UF strains. This enabled us to analyse crosses by plating on non-selective media. Table 4 shows the genotypes of the progeny from the Rcg3 × A585 cross. Unlike the situation in an NF × UF cross of *S. coelicolor* (Hopwood *et al.*, 1969), many clones had the parental genotypes (83/240 with the Rcg3 genotype and 4/240 with strain A585 genotype) and there was a distinct ratio of recombinant classes. Figure 3*a* shows the allele ratios of the 153 clones with recombinant genotypes: these ratios depart markedly from those expected from crosses between *S. coelicolor* NF and UF strains. Of 153 recombinants analysed, 145 had the phenotypic characteristic of SCP1-carrying strains in arresting the development of *S. coelicolor* SCP1$^-$ strains. In a qualitative test for the ability to generate recombinants with one of the UF tester strains A585, A617 or S32, representatives of recombinants from this cross behaved like the donor strain of *S. coelicolor*, i.e. they produced recombinants with a high frequency, unlike the situation in the cross with an IF strain of *S. coelicolor* A3(2). As in all well known conjugational systems, effective transfer of the chromosome from Rcg3 into the UF strain of *S. coelicolor* may be due to the presence of fertility factor in this strain. For operational convenience we tentatively named this SGP1 in Fig. 3*a*.

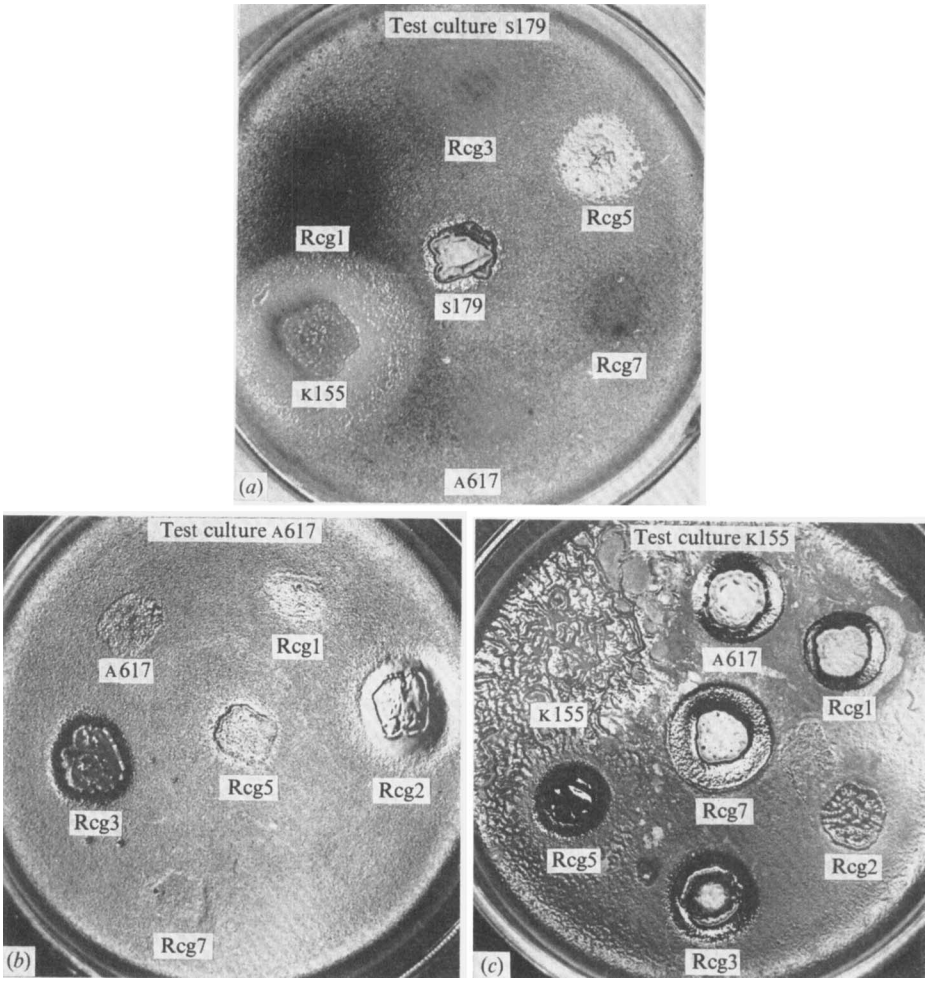

Fig. 2. Inhibition of (*a*) *S. coelicolor* S179 NF, (*b*) *S. coelicolor* A617 UF and (*c*) *S. griseus* K155 by recombinants from crosses between *S. coelicolor* and *S. griseus*. (*a*) Only strain K155 inhibits the NF strain (light zone). (*b*) Only recombinants Rcg2 and Rcg3 inhibit the UF strain. Rcg3 forms dark zones of inhibition because patches produce dark pigment during growth. Rcg2 forms light zones (dark pigment is not produced). (*c*) The test culture K155 is inhibited by strains A617, Rcg1, Rcg3, Rcg5 and Rcg7.

Table 3. *Cross-testing of antibiotic-producing strains of S. coelicolor* A3(2) *and S. griseus* Kr.15 *and their recombinants*

	Indicator cultures								
Strains	A617 UF	S179 NF	K806	K155	Rcg1	Rcg3	Rcg7	Rcg2	Rcg11
A617, Rcg1, Rcg5, Rcg7	−	−	+	+	−	−	−	+	+
S179, Rcg3	+	−	+	+	+	−	+	+	+
K806, K155, Rcg2, Rcg11	+	+	−	−	+	+	+	−	−

+, Inhibition; −, no inhibition.

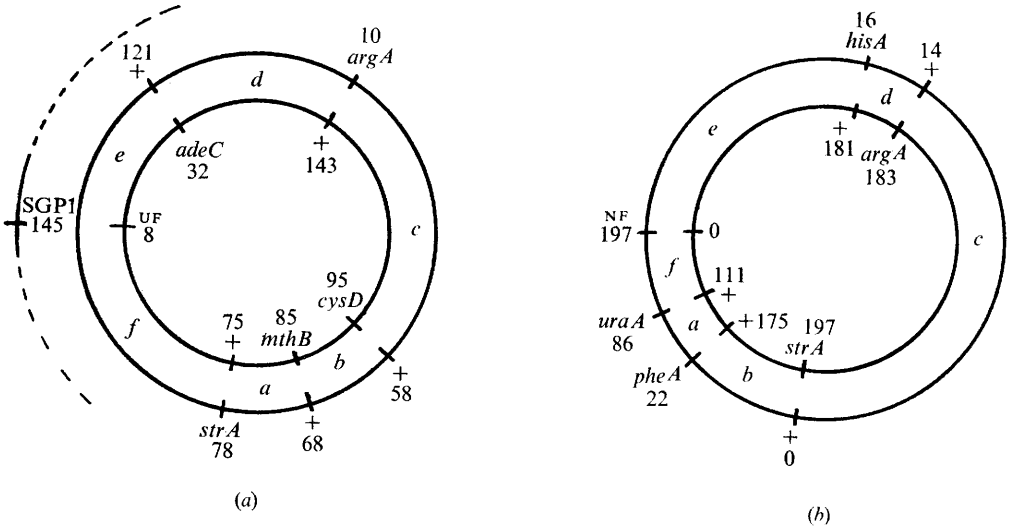

Fig. 3. Non-selective analysis of genotypes of haploid recombinants from the crosses (*a*) Rcg3 × A585, parental genotypes omitted, and (*b*) S179 × Rcg1.

Table 4. *Non-selective analysis of genotypes from crosses* Rcg3 × A585 *and* S179 × Rcg1*

Genotype	Crossover in interval	No. of variants	Fertility type
Cross Rcg3 *arg str* × A585 *ade mth cys*			
ade mth cys	—	4	UF
mth cys	*d, e*	8	Recipient-like
arg str	—	83	Donor-like
mth cys	*d, f*	29	Donor-like
str	*c, d*	48	Donor-like
mth cys str	*a, d*	14	Donor-like
mth cys ade	*e, f*	24	Donor-like
cys str	*b, d*	5	Donor-like
arg cys str	*b, c*	4	Donor-like
ade mth cys str	*a, e*	3	Donor-like
arg mth cys	*c, f*	2	Donor-like
arg	*a, f*	1	Donor-like
arg mth	*b, f*	1	Donor-like
ade cys str	*b, e*	1	Donor-like
Prototroph	*a, c, d, f*	5	Donor-like
ade arg mth cys	*c, d, e, f*	2	Donor-like
ade cys	*a, b, e, f*	1	Donor-like
mth str	*a, b, c, d*	1	Donor-like
ade arg mth cys str	*a, c, d, e*	1	Donor-like
mth	*b, c, d, f*	1	Donor-like
cys	*a, b, d, f*	1	Donor-like
ade mth str	*a, b, c, e*	1	Donor-like
Cross S179 *his ura phe* × Rcg1 *arg str*			
arg str	*e, f*	110	NF
ura arg str	*a, e*	58	NF
ura phe his str	*b, c*	11	NF
ura phe arg str	*b, e*	11	NF
ura arg his str	*a, d*	4	NF
arg his str	*d, f*	1	NF
str	*c, d, e, f*	1	NF
ura str	*a, c, d, e*	1	NF

* See Fig. 3 (*a*, *b*).

The behaviour of Rcg1 in crosses with *S. coelicolor* NF strains was similar to that of a UF strain. The results of non-selective genetic analysis of recombinants from the cross between *S. coelicolor* NF and Rcg1 suggest that Rcg1 acts as a recipient. All the progeny, including variants with the genotype of a UF strain, possessed the phenotypic characteristic of SCP1 plasmid-carrying strains (Table 4, Fig. 3*b*) and in crosses with UF tester strains behaved as donor strains.

Recombinants carrying different parental contributions

The results presented above demonstrate that recombinants between *S. coelicolor* and *S. griseus* were not easily detected in crosses conducted in the presence of streptomycin. The following technique was found to be more appropriate. Spores of both parents were mixed and inoculated on agar, and the resulting young mixed culture was transferred to medium containing streptomycin at various times before the antibiotic produced by the donor strain prevented further development of the recipient strain. Colonies were readily recognizible on selective medium when the mixed cultures were transferred after incubation for 6 to 18 h, but the number of such colonies was markedly reduced if the mixed culture was transferred after longer incubation times. The largest number of colonies was obtained when parental mixtures were transferred after 10 h. In the cross K806 × S18 (selected markers *adeC*$^+$ *strA1*), colonies were formed at a frequency of 2×10^{-3} to 5×10^{-3} on selective plates, but when transferred to the same selective plates, they failed to grow. Twenty such colonies were seeded on CM. Only variants with the genotype of the parental strain S18 were detected among 100 progeny analysed from each of 17 colonies. Among the progeny from the other three colonies (Table 5), segregants *ura mth cys str* and *str* were detected as well as those with the genotype of the parental strain S18. *Str* recombinants and strain K806 produced similar amounts of grisin. A representative of this recombinant class was designated Rcg11.

To construct recombinants carrying different contributions from the parental *S. griseus* chromosome, Rcg1 was crossed with *S. griseus* K155. *ArgA1*$^+$ and *strA1* served as selected markers. Genetic exchange between these strains may occur more readily because the parental chromosomes had *S. griseus* chromosomal fragments of complete homology. Colonies were detected on selective media at a frequency of approximately 5×10^{-1} and failed to grow when transferred to the same media. Recombinants with new combinations of chromosomal markers were found among the progeny of three out of seven such colonies when about 100 clones from each colony were analysed (Table 6). The inhibiting properties of representatives of recombinant classes are shown in Table 3. Rcg5 and Rcg7 have the same properties as the parental strain Rcg1. In contrast, Rcg2 produced the antibiotic that specifically inhibits *S. coelicolor* SCP1-carrying and SCP1$^-$ strains but not *S. griseus* K155. While growing in a liquid medium, Rcg2 produced grisin at a level similar to that of the parental strain K155.

Host–phage relationships

Recombinants were used to determine the host range of the two groups of actinophages, each acting specifically on one of the parental strains and being unable to adsorb to the other (Tables 7 and 8). Phages ϕ448 and VP5 were able to adsorb to and grow in strains of *S. coelicolor* and *S. lividans*. Phages Pg81, Pg100 and Pg56 behaved in *S. griseus* K806 host in the same manner. Strain K155 was resistant to phage Pg81: though able to adsorb to this strain, the phage failed to produce plaques. Rcg1, Rcg3 and Rcg5 were similar to *S. coelicolor* in respect of all phages examined. Rcg7, Rcg2 and Rcg11 differed from both parental strains in their ability to adsorb and support phage growth. Phage Pg81 on lawns of Rcg2

Table 5. *Genotypes of the progeny of colonies formed on MM from the cross between strains* K806 *and* S18

Colony no.	No. of progeny analysed with genotype: *ade ura mth cys str*	*ura mth cys str*	*str*
1	82	9	0
9	98	12	0
11	104	0	14

Table 6. *Genotypes of the progeny of colonies formed on MM from the cross between strains* Rcg1 *and* K155

Colony no.	No. of progeny analysed with genotype: *argA1 strA1*	*strA1*	*argA1 his-1 strA1*
2	69	16	0
5	116	0	4
7	15	125	0
9	150	0	0
10	125	0	0
11	132	0	0
12	111	0	0

Table 7. *Efficiency of actinophage adsorption to actinomycete strains*

	Efficiency of adsorption (%)									
	Indicator and parental strains				Recombinants					
Phages	66	A617, S18	K806	K155	Rcg1	Rcg3	Rcg5	Rcg7	Rcg2	Rcg11
ϕ448	48	51	< 0·001	< 0·001	55	58	53	48	< 0·001	< 0·001
VP5	54	49	< 0·001	< 0·001	52	54	60	52	58	50
Pg81	< 0·001	< 0·001	58	50	< 0·001	< 0·001	< 0·001	< 0·001	51	49
Pg100	< 0·001	< 0·001	52	51	< 0·001	< 0·001	< 0·001	< 0·001	50	52
Pg56	< 0·001	< 0·001	62	60	< 0·001	< 0·001	< 0·001	59	58	60

Table 8. *Efficiencies of actinophage plating on actinomycete strains*

					Recombinants from crosses				
	Indicator and parental strains				A617 × K806	K155 × Rcg1			S18 × K806
Phages	66	A617, S18	K806	K155	Rcg1, Rcg3	Rcg5	Rcg7	Rcg2	Rcg11
ϕ448	1·0	1×10^{-1}	—*	—	1×10^{-1}	1×10^{-1}	$< 1 \times 10^{-1}$	—	—
VP5	1·0	1·0	—	—	1·0	1·0	1·0	$< 1 \times 10^{-7}$	$< 1 \times 10^{-7}$
Pg81	—	—	1·0	$< 1 \times 10^{-9}$	—	—	—	1×10^{-4}	3×10^{-5}
Pg100	—	—	1·0	1·0	—	—	—	$< 1 \times 10^{-8}$	$< 1 \times 10^{-8}$
Pg56	—	—	1·0	1×10^{-1}	—	—	$< 5 \times 10^{-7}$	1×10^{-1}	1·0

* —, Phage failed to adsorb to the culture.

Table 9. *Efficiencies of plating of* Pg81 *on strains* K806, Rcg2 *and* Rcg11

Host strain	Relative e.o.p. of phage Pg81 lysates prepared from strains: K806	Rcg2	Rcg11
K806	1	1×10^{-7}	3×10^{-7}
Rcg2	1×10^{-4}	1	1
Rcg11	3×10^{-5}	1	1

and Rcg11 gave plaques as large as on *S. griseus* K806 though with lower efficiencies of plating (e.o.p), 1×10^{-4} and 3×10^{-5}. Phages from large plaques when growing in Rcg2 or Rcg11 gave a high e.o.p. on these strains and were restricted in *S. griseus* K806 (Table 9).

DISCUSSION

Under conditions that reduced the effect of antibiotic inhibition (in the presence of streptomycin, or after transfer of young mixed culture to a medium containing streptomycin), colonies with the properties of heteroclones arose on selective plates from crosses between *S. griseus str-s* and *S. coelicolor* UF (SCP1$^-$) *str-r* strains: they were unable to grow on the same medium and could generate heterogeneous progeny. Despite the fact that in the progeny of these colonies most variants had the genotype of *S. coelicolor*, some variants which differed from both parental strains in their genotypes were also detected. However, only a limited number of recombinant classes were revealed in the progeny of such colonies unlike the situation when heteroclones formed in crosses between *S. coelicolor* strains were analysed. Infrequent crossing-over between the chromosomes of *S. coelicolor* and *S. griseus* may be explained by imperfect homology, or the possibility that heteroclones and recombinants generated in crosses between A3(2) derivatives are structurally different from those formed in the cross between *S. coelicolor* and *S. griseus*. The latter may possess a fragment of the *S. griseus* chromosome that is not integrated into the chromosome of *S. coelicolor*. Such extrachromosomal elements may be lost or preserved in heteroclone progeny. The cells containing such fragments phenotypically may resemble haploid recombinants.

Characteristics of representatives of the recombinant classes were studied. Some recombinants failed to produce the antibiotic that inhibits SCP1-carrying and SCP1$^-$ strains of *S. coelicolor*. Rcg1 resembled *S. coelicolor* SCP1$^-$ strains in its inability to arrest SCP1-carrying strains and in its sensitivity to the antibiotic produced by SCP1-carrying strains. In crosses with *S. coelicolor*, the SCP1-carrying NF strain Rcg1 behaved like a recipient. Only recombinant progeny were produced in these crosses. Zygotes from such crosses are polarized and asymmetric since the chromosomal fragment is derived from the *S. coelicolor* NF strain. In crosses between *S. griseus* strain K155 and Rcg1, colonies having properties of heteroclones arose at a high frequency. Rcg3 phenotypically resembled a SCP1-carrying strain in its ability to inhibit only SCP1$^-$ strains and in its resistance to the antibiotic produced by SCP1-carrying *S. coelicolor* strains. In crosses between Rcg3 and a *S. coelicolor* UF strain, recombinants appeared at high frequency. However, unlike *S. coelicolor* NF × UF crosses, variants with parental genotypes were detected in the progeny and a distinct ratio of recombinant classes was observed. The reason for this phenomenon is not obvious. The differences could be related to an anomaly imposed by the absence of crossovers between chromosomes of Rcg3 and strain A585 in a region of Rcg3 containing

a fragment of the *S. griseus* chromosome with the *uraA1*$^+$ marker. For example, recombinants formed by crossovers in regions *a*, *b* and *c* (Fig. 3) and rarely detected in the progeny from crosses between A3(2) strains, may be revealed more often in the progeny from the cross Rcg3 × A585. However, other explanations must also be considered.

Donor properties of *S. griseus* are indicated by the isolation from crosses between *S. griseus* and a *S. coelicolor* recipient strain of the recombinant Rcg3 which had donor strain properties, and by the effective transfer of *S. griseus* chromosomal genes into Rcg1. Also, Rcg3 was able to arrest the growth of SCP1$^-$ strains of *S. coelicolor*. This donor ability may be related to the presence in Rcg3 and *S. griseus* of a plasmid resembling SCP1.

Our results also demonstrate the haploid nature of the isolated recombinants. All of them, Rcg1, Rcg3, Rcg11 derived from crosses between *S. griseus* and *S. coelicolor* and Rcg2, Rcg5 and Rcg7 from crosses between *S. griseus* and Rcg1, were stable and did not segregate clones of the recipient genotype. In addition, the functioning of the recessive allele of the donor for histidine requirement is expressed in Rcg5, and the other donor character, i.e. the absence of the ability to adsorb actinophage ϕ448 (that may be considered as a recessive character provided the recipient strain is able to adsorb this phage) is expressed in Rcg2 and Rcg11. Further studies are needed to determine whether stable integration of a fragment of the *S. griseus* chromosome into the chromosome of *S. coelicolor* takes place. Rcg1, Rcg3 and Rcg5 were, like *S. coelicolor*, hosts for a number of phages, whereas Rcg2, Rcg7 and Rcg11 differed from both parents in this respect. The behaviour of Rcg1, Rcg3 and Rcg5 suggests that they lack genes controlling phages Pg81, Pg100 and Pg56 receptor synthesis and that genes essential for ϕ448 and VP5 adsorption and growth are located in the fragment of these recombinants which was derived from the *S. coelicolor* chromosome. Recombinant Rcg7, unlike *S. coelicolor*, acquired the gene for adsorption of actinophage Pg56. Since the growth of phage Pg56 is limited in Rcg7, we take this to mean that it is connected with the absence in the recombinants of those genes of the *S. griseus* chromosome involved in the control of intracellular phage growth. Alternatively, it is possible that the gene responsible for Pg56 growth limitation is located in the particular fragment of the *S. coelicolor* chromosome contributed to Rcg7. The genes for ϕ448 and VP5 adsorption and those responsible for phage growth may be located in the same fragment.

Rcg2 and Rcg11 contain genes responsible for adsorption of phages Pg81, Pg100, Pg56 and VP5. However, only phage Pg56 was capable of growing in Rcg2 and Rcg11. When plated on Rcg2 and Rcg11, phage Pg81 gave rise to modified phage, the latter producing high yield in these hosts. The phage was also restricted in *S. griseus*. Thus, we assume that *S. coelicolor* has a restriction and modification system. The data also imply that two different systems of restriction and modification of phage DNA exist in *S. coelicolor* and *S. griseus*.

The growth limitation of phages Pg100 and VP5 in Rcg2 and Rcg11 strains may be the result of the absence in hybrids of genes essential for phage intracellular growth, or the presence of host genes acting by a mechanism distinct from that for restriction and modification. The data presented show the possibility of obtaining hybrids with desired characteristics, for example, strains that produce grisin and are resistant to phages which lyse industrial grisin-producers. In the course of these studies we obtained recombinants (Rcg2 and Rcg11) which were able to produce grisin under conditions of liquid growth in amounts similar to those of the parental strains, *S. griseus* K155 and K806. They were also resistant to phage ϕ448 and restricted phages VP5, Pg81 and Pg100. Thus they did not support growth of two phages acting on *S. coelicolor*, and two acting on *S. griseus*.

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