Genetic Evidence for a Plasmid Controlling Fertility in an Industrial Strain of *Streptomyces rimosus*

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A plasmid (SRP1) determines most of the genetic recombination which occurs between marked derivatives of our strain of *Streptomyces rimosus*. SRP1⁻ strains occur with a frequency of about 0.3% amongst spores of SRP1⁺ strains. In SRP1⁺ × SRP1⁻ crosses, SRP1 is transferred to more than 50% of the SRP1⁻ parent. SRP1⁺ × SRP1⁻ crosses are about 100 times more fertile than SRP1⁺ × SRP1⁺ crosses (10^{-3} to 10^{-4} compared with 10^{-5} to 10^{-7}). A very low frequency (about 10^{-8}) of true recombinants occurs in the absence of SRP1.

INTRODUCTION

Crosses between auxotrophically marked strains derived either from a wild-type culture of *Streptomyces rimosus* or from mutants of it producing higher titres of oxytetracycline yield recombinants at frequencies between 10^{-5} and 10^{-7} (Friend & Hopwood, 1971; E. J. Friend and M. Warren, unpublished results). In the hope of discovering strains of enhanced fertility, which might occur amongst recombinants, the fertility of each cross performed in routine genetic work was recorded. Eventually a cross was encountered which gave about 100 times more recombinants than usual and this behaviour was ascribed to one particular parent in the cross. The characteristics and inheritance of the enhanced fertility character were those expected if this strain had arisen by loss of a sex factor. We have called the fertility plasmid SRP1 (*Streptomyces rimosus* plasmid 1) by analogy with *Streptomyces coelicolor* A3(2) in which two sex factors, SCP1 and SCP2, have been identified (Vivian, 1971; Bibb, Freeman & Hopwood, 1977).

METHODS

General. Complete medium and standard cultural and crossing techniques were those described by Friend & Hopwood (1971). Minimal medium contained the following ingredients per litre of distilled water: FeSO₄·7H₂O, 0.01 g; MgSO₄·7H₂O, 0.2 g; K₂HPO₄, 0.5 g; sodium glutamate, 0.5 g; NaNO₃, 0.8 g; glycerol, 10 g; agar, 20 g. Minimal medium was adjusted to pH 7.0 with HCl before sterilization.

Strains. These were derived by mutation and recombination from a prototrophic culture used commercially for the production of oxytetracycline. The linkage relationships and characteristics of auxotrophic loci were described by Friend & Hopwood (1971).

Measurement of fertility. Unless otherwise stated, the fertility of a slant cross was the total number of recombinants recoverable on two selective media as a fraction of the total viable units from the cross.

Isolation of fertility variants. Spontaneous fertility variants were isolated by replica-plating a population of SRP1⁺ colonies (100 to 200 per plate) from one strain on to plates of complete medium spread with a dense spore suspension ('lawn') of a second strain, carrying complementary auxotrophic markers. The

Fig. 1. The fertility difference between $SRP1^+$ and $SRP1^-$ strains in plate-crosses.

Top: Cultures isogenic for chromosomal markers *ribB1 lysB2*: r925 $SRP1^-$ (left) and r289 $SRP1^+$ (right).

Centre: Plate-crosses between top plate and 'lawns' of strains isogenic for *adeC2 cysD3 ribB1*: r723 $SRP1^+$ (left-hand plate) and r559 $SRP1^-$ (right-hand plate).

Bottom: Replicas from centre plates to medium selecting ade^+lys^+ recombinants. From left to right the results on each half-plate are as follows: (1) $SRP1^- \times SRP1^+$, each original colony has produced numerous recombinants; (2) $SRP1^+ \times SRP1^+$, occasionally an original colony has yielded a single recombinant; (3) $SRP1^- \times SRP1^-$, no recombinants; (4) duplicate of (1) with fertility of partners reversed.

resulting 'plate-crosses', after incubation for 5 d at 30 °C, were replicated to a medium selecting recombinants. SRP1⁻ colonies produced numerous recombinants, whereas the majority of colonies gave no recombinants or, more rarely, one or two. The procedure was essentially the same as that used by Hopwood *et al.* (1969) to isolate SCP1⁻ variants of *S. coelicolor*.

Analysis of recombinants from SRP1⁺ × SRP1⁻ crosses. Identification of spontaneous SRP1⁻ variants made it possible to isolate pairs of strains isogenic for auxotrophic markers but of different fertility type. These pairs provided useful 'tester' strains for determining the fertility type of recombinants recovered from SRP1⁺ × SRP1⁻ crosses. A new recombinant, depending on its fertility type, gave a high recombination frequency with one tester (a patch of recombinants in a plate-cross test) and a negative result with the other (few or no recombinants in a plate-cross test) (Fig. 1). Recombinants were recovered on at least two selective media and were purified by re-streaking twice on the medium used to select them. Because recombinants had been purified, thus avoiding contamination from the parent strains, it was possible to use complete medium for the preparation of master plates, accommodating 50 colonies per plate. These plates gave better sporulation of the recombinant cultures than did supplemented minimal medium and this facilitated further testing by replication of master plates to diagnostic media to determine auxotrophic phenotype and to lawns of SRP1⁺ and SRP1⁻ testers for fertility type.

RESULTS

Recognition of an enhanced fertility variant and its characterization as an SRP1⁻ strain

The number of recombinants selected from a standard crossing slant was used as an estimate of the fertility of each cross in a large series involving many different strains, most of which were recombinants derived from earlier crosses. One cross yielded approximately 100 times more recombinants than usual. The enhanced fertility of this cross was associated with one parent, R559, because this strain had higher fertility with five different partners chosen at random from the culture collection, while the other parent behaved normally.

Until the discovery of the special behaviour of strain R559, a cross made by replicating colonies of one parent to a lawn of the other on non-selective medium and subsequently printing this plate-cross to selective media produced few recombinants, each isolated colony of the tested strain yielding one or two recombinant colonies at most. In contrast, each replicated colony of R559 yielded numerous recombinants in such a plate-cross.

In principle, the enhanced fertility of strain R559 could have resulted from a chromosomal mutation or from loss of, integration of or mutation in a plasmid. To distinguish between these possibilities, a cross of strain R559 *adeC2 cysD3 ribB1* with a strain of normal fertility, R276 *hisA1*, was plated on selective media to recover recombinant and parental colonies (Table 1). Samples of each parental phenotype emerging from the cross were tested for fertility by plate-crossing with normal strains. None of a sample of 100 colonies of the R276 parental phenotype was changed in fertility, but 54% of colonies carrying the markers of R559 differed from it in having the normal low fertility, while the remaining 46% had the higher fertility of R559. This result indicated transfer of fertility type at a frequency some 2000 times higher than chromosomal marker exchange, suggesting plasmid involvement. Moreover, the infectious conversion of R559 to normal fertility rather than of R276 to the variant type suggested that R559 had lost a sex factor, rather than having acquired a variant form of it. We therefore designated R559 as an SRP1⁻ strain.

Origin of new SRP1⁻ strains

Colonies of several different strains were tested for fertility by the plate-crossing technique, against strains of the usual fertility type. Higher fertility variants comparable with strain R559 were found in each strain at between 0.2 and 0.8% of the population (Table 2). The high spontaneous frequency with which such variants arose in untreated cultures was consistent with the hypothesis of plasmid loss to account for their origin.

Table 1. Analysis of a cross between R559 *adeC2 cysD3 ribB1* and R276 *hisA1*

Total recombinants recovered on three selective media	3.2×10^6
Auxotrophic parental phenotype His ⁻ , low fertility	3.6×10^9
*Auxotrophic parental phenotype Ade ⁻ Cys ⁻ Rib ⁻ , high fertility	5.3×10^9
*Auxotrophic parental phenotype Ade ⁻ Cys ⁻ Rib ⁻ , low fertility	6.2×10^9
Total plating units recovered from the cross	1.5×10^{10}
Fraction of chromosomal recombinants	2.0×10^{-4}
Frequency of transfer of fertility determinant	5.4×10^{-1}

* The total Ade⁻Cys⁻Rib⁻ colonies were classified on the basis of an analysis of 254 colonies, 117 of which (46.1%) had the high fertility of the original R559 parent when plate-crossed with R276 while 137 (53.9%) had low fertility.

Table 2. Origin of spontaneous SRP1⁻ variants

Strain	Total no. of colonies	SRP1 ⁻ variants	
		Number	Percentage
R289 <i>ribB1 lysB2</i>	1018	3	0.3
R876 <i>proA3 uraA8 argA1 cysD3</i>	924	7	0.8
R723 <i>adeC2 cysD3 ribB1</i>	2331	4	0.2
Total/Average	4273	14	0.3

Table 3. Classification of recombinants from an SRP1⁺ × SRP1⁻ cross: R279 SRP1⁺ *argA1 adeC2 lysB2* × R689 SRP1⁻ *proA3 uraA8 cysD3**

Selected markers <i>arg</i> ⁺ / <i>ura</i> ⁺				Selected markers <i>lys</i> ⁺ / <i>pro</i> ⁺			
Genotype	SRP1 ⁺	SRP1 ⁻	Total	Genotype	SRP1 ⁺	SRP1 ⁻	Total
<i>cys</i>	1	0	1	<i>cys ura</i>	8	5	13
<i>pro cys</i>	27	3	30	<i>cys ade</i>	25	2	27
<i>ade cys</i>	6	0	6	<i>arg ura</i>	9	0	9
<i>pro ade cys</i>	40	8	48	<i>cys ade arg</i>	17	4	21
<i>pro lys cys</i>	1	0	1	<i>cys arg ura</i>	22	2	24
Total	75	11	86	Total	81	13	94

* The markers *adeC2* (requirement for purines) and *uraA8* (requirement for uracil) were added to the map after publication of linkage data for *S. rimosus* (Friend & Hopwood, 1971). The convention adopted previously for assigning locus letters was retained, i.e. letters were chosen not to conflict with the known loci of *S. coelicolor*.

Table 4. Fertility of crosses of differing fertility type

Frequencies represent the proportion of selected recombinants amongst the total output of the crosses. Each frequency is the average for two selections.

SRP1 ⁺ × SRP1 ⁻	SRP1 ⁺ × SRP1 ⁺	SRP1 ⁻ × SRP1 ⁻
1.7 × 10 ⁻³	1.2 × 10 ⁻⁵	1.4 × 10 ⁻⁷
1.0 × 10 ⁻³	5.3 × 10 ⁻⁶	1.9 × 10 ⁻⁸
7.1 × 10 ⁻⁴	2.0 × 10 ⁻⁶	7 × 10 ⁻⁹
1.4 × 10 ⁻⁴	1.3 × 10 ⁻⁶	5 × 10 ⁻⁹
1.3 × 10 ⁻⁴	4.5 × 10 ⁻⁷	< 2 × 10 ⁻¹⁰
1.2 × 10 ⁻⁴	1.6 × 10 ⁻⁷	< 2 × 10 ⁻¹⁰
1.1 × 10 ⁻⁴	1.0 × 10 ⁻⁷	
Average 5.6 × 10 ⁻⁴	3.1 × 10 ⁻⁶	2.8 × 10 ⁻⁸

Segregation of fertility type in SRP1⁺ × SRP1⁻ crosses

Results from a typical SRP1⁺ × SRP1⁻ cross are shown in Table 3. Although the fertility difference of the two parents segregated amongst the recombinants, it proved impossible to establish a map location for the fertility determinant compatible with the segregation of standard markers when different selection patterns were employed. With selection of Arg⁺ and Ura⁺, the fertility determinant could have been located, on the basis of allele ratios, between *lysB* and *uraA* or between *uraA* and *adeC*; with selection of Pro⁺ and Lys⁺, the positions were between *adeC* and *proA* or between *proA* and *argA*. Such crosses excluded a map location for the fertility determinant, supporting the earlier evidence which indicated plasmid involvement in determining the fertility of *S. rimosus* crosses.

Fertility of crosses of different SRP1 status

Earlier experiments to assess the fertility of crosses relied solely on estimates of total recombinants recovered from one standard crossing slant and indicated that SRP1⁺ × SRP1⁻ crosses were, on average, more fertile than SRP1⁺ × SRP1⁺ crosses by a factor of about 100. A more detailed study of crosses involving strains of different SRP1 status was now required in order to measure the fertility of crosses with greater precision and to provide data directly comparable with data for *S. coelicolor*.

Three series of slant-crosses were made, each comprising six or seven crosses, of SRP1⁺ × SRP1⁻, SRP1⁺ × SRP1⁺ or SRP1⁻ × SRP1⁻ type respectively. The fertility of these crosses is given in Table 4. Although there was considerable variation in fertility within each series of crosses, the three series were clearly of different fertility. SRP1⁺ × SRP1⁻ crosses were two orders of magnitude more fertile, on average, than SRP1⁺ × SRP1⁺ crosses, which were a further two orders of magnitude more fertile than SRP1⁻ × SRP1⁻ crosses.

Colonies recovered on selective media from SRP1⁻ × SRP1⁻ crosses could have arisen by reversion of one marker in either parent strain, since their numbers were within the expected frequency range. However, proof that some of these colonies, at least, were genuine recombinants was provided by their inheritance of auxotrophic markers from each parent.

DISCUSSION

The results reported here implicate a sex factor (SRP1) as the primary agent promoting gene exchange in our strain of *S. rimosus*. The genetic evidence for its existence is similar to that which served to identify the two sex factors of *S. coelicolor* A3(2), of which one, SCP2, has also been characterized physically (Bibb *et al.*, 1977). No attempt has yet been made to isolate plasmid DNA corresponding to SRP1.

In *S. coelicolor* A3(2), the fertility properties of the two sex factors differ in the following

way. While $SCP1^+ \times SCP1^-$ crosses are somewhat less fertile than $SCP1^+ \times SCP1^+$ crosses, $SCP2^+ \times SCP2^-$ crosses are always at least one order of magnitude, and often two orders, more fertile than $SCP2^+ \times SCP2^+$ crosses (Bibb, 1977). In other words, the presence of SCP2 in a strain tends to limit the transfer into it, or effective inheritance from it, of genetic markers from another strain carrying the same sex factor, while the presence of SCP1 does not. To what extent this effect can be explained by phenomena such as surface exclusion and plasmid incompatibility, described for eubacterial plasmids, has not yet been elucidated because of problems associated with the mycelial growth habit of streptomycetes. A variant form of SCP2, called $SCP2^*$, has been recognized, and it resembles SCP1 in that the fertility of $SCP2^* \times SCP2^*$ crosses is not reduced compared with that of $SCP2^* \times SCP2^-$ crosses (Bibb, 1977). The present results indicate that SRP1 resembles SCP2 rather than SCP1 or $SCP2^*$ in respect of this phenomenon, since $SRP1^+ \times SRP1^-$ crosses were 100 times more fertile than $SRP1^+ \times SRP1^+$ crosses.

Another difference concerns the proportion of $SRP1^+$ progeny arising from an $SRP1^+ \times SRP1^-$ cross. In *S. coelicolor*, virtually all $SCP1^-$ or $SCP2^-$ parental genotypes are converted during the course of a cross with $SCP1^+$ or $SCP2^+$ strains to the plasmid carrying type, and virtually all recombinants are found to possess the plasmid. In contrast, a considerable fraction of the $SRP1^-$ parental type was not converted to $SRP1^+$ in crosses with $SRP1^+$ strains, and significant proportions of $SRP1^-$ recombinants were also produced.

As in the case of $SCP1^- \times SCP2^-$ crosses, $SRP1^- \times SRP1^-$ crosses are not completely sterile. This finding perhaps supports the idea that a low level of gene exchange in streptomycetes can occur in the absence of any sex factor. The alternative hypothesis demands the existence of at least one further sex factor in both *S. coelicolor* A3(2) and *S. rimosus*.

Perhaps the main importance of the present results is the demonstration of the involvement of a plasmid sex factor in conjugation leading to gene exchange in a second streptomycete, suggesting that this may be the explanation for appreciable frequencies of recombination in most *Streptomyces* spp. Although recombination has been described in many other streptomycetes (Hopwood & Merrick, 1977), including another strain of *S. rimosus* in which extensive mapping studies have been carried out (Alačević, 1976), sex factors have not yet been implicated in recombination in these strains.

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