

Infectious transfer of a fertility factor in *Streptomyces coelicolor*

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SUMMARY

Initial Fertility (IF) strains of *Streptomyces coelicolor* are able to convert recipient strains (UF) to the IF condition by contact, without concomitant transfer of chromosomal markers. The conversion is prevented by the presence of acridine orange in the medium of the mixed culture. Acridine orange is also moderately effective in inducing the formation of UF variants from IF-treated strains. No effect of the drug is observed on UF variant formation from Normal Fertility (NF) strains nor on the behaviour of the fertility factor in NF × UF mixed cultures. The hypothesis is put forward that the fertility factor works as an episome in *S. coelicolor*, fixed to the chromosome in the NF strains, free in the IF strains and missing in the UF strains.

1. INTRODUCTION

The recent work on the fertility system of *Streptomyces coelicolor* A3(2) (Hopwood *et al.* 1969; Sermonti, Puglia & Ficarra, 1971; Culotta & Puglia, 1972) clearly points to the occurrence in some NF strains of a fertility factor located on the circular map of the species at the conventional 9 o'clock position. The occurrence of a chromosomal factor does not, however, explain all the reported results. While the discovery of very effective recipient strains (UF) by Hopwood *et al.* (1969) can well be interpreted as due to the loss of the factor, the intermediate class with moderate donor and recipient ability (IF) also described by Vivian & Hopwood (1970) cannot be fitted in to this simple 'all or none' model. The very effective recipients (UF) and the very effective donors (NF) both trace back to the IF types (Vivian & Hopwood, 1970). While UF strains are easily obtained from IF strains, either spontaneously or after treatment with u.v. or X-rays (Hopwood *et al.* 1969; Vivian & Hopwood, 1970), NF strains have so far only been found in the stock cultures, and no means for inducing or selecting them from IF strains has been reported. The NF strains appear unaffected by treatment with u.v. or X-rays (Vivian & Hopwood, 1970) but they are able after a short contact to convert UF cells to IF (Sermonti *et al.* 1971). The easy removal of the fertility factor from the IF cells, the rapid conversion of UF cells to IF after contact with NF cells, as well as the very low donor ability of the IF strains, all point to a peculiar situation of the F factor in the latter group of strains.

The present paper reports a series of results which clearly indicate the extra-chromosomal seat of the fertility factor in at least some of the IF strains. The factor appears thus to belong to the class of elements termed 'episomes' (Jacob & Wollman, 1958).

2. MATERIALS AND METHODS

(i) *Strains and culture conditions*

All the mutant strains used throughout the work trace back to the strain *Streptomyces coelicolor* A3(2) described by Hopwood & Sermonti (1962). Most of the strains used were obtained from Hopwood's collection of strains, but their fertility pattern was modified (either spontaneously or deliberately) and determined in the authors' laboratory. A list of the strains employed is given in Table 1. The culture media and the crossing procedures are the same as those described in previous papers (Hopwood & Sermonti, 1962; Sermonti *et al.* 1971).

(ii) *Fertility type*

As shown in Table 1, the strains adopted belong to one of the fertility groups described by Hopwood *et al.* (1969) and by Vivian & Hopwood (1970) as NF, UF or IF. The present work deals mainly with IF and UF strains, and their mutual

Table 1. *List of strains employed in the present work*

Code no.	Fertility type	Genotype*
39	NF	<i>hisA1</i>
44	IF	<i>hisA1</i>
321	UF	<i>hisA1</i>
326	NF	<i>argA1 uraA1 strA1</i>
118-4	IF	<i>argA1 uraA1 strA1</i>
312	UF	<i>argA1 uraA1 strA1</i>
80	NF	<i>argA1 hisA1 leuA1 uraA1 strA1</i>
316	UF	<i>pheA1 strA1 hisD3</i>

* *arg*, *his*, *leu*, *phe*, *ura*: requirements, respectively, for arginine, histidine, leucine, phenylalanine, uracil. *str*: resistance to streptomycin.

conversion. The two types are easily distinguished by mating to an NF tester strain, IF strains being much poorer than UF as recipients. This holds particularly in our experimental conditions, the recipient ability of the IF strains adopted being from 1000 to 100 times lower (versus an NF tester) than that of the UF strains.

3. RESULTS

(i) *Recombination frequency in crosses involving different fertility types*

UF, IF and NF fertility variants have been obtained from strains of two genotypes: *his A1* and *argA1 ura A1 strA1*. They have been crossed in all possible combinations and spores of mixed cultures plated on three media, selecting for

recombinants (on minimal medium + arginine) and for either parent phenotype (Table 2).

NF × UF mixed cultures produce spores which are virtually all derived from segregation processes (Hopwood *et al.* 1969; Sermonti *et al.* 1971). NF × NF crosses are also fertile, but at a much lower level. The recombination frequency ranges between 10⁻² and 10⁻³ referred to the minority parent. UF × UF crosses are virtually sterile. We have never recorded recombination frequencies higher than 10⁻⁶, but Vivian & Hopwood (1970) report a ratio of 2.9 × 10⁻⁴ of selected recombinants to minority parental type in one such cross. The NF strains can be tentatively regarded as high-frequency donors, and the UF strains as most effective recipients.

Table 2. *Frequency of selected recombinants in crosses involving strains of various fertility types*

Strains				Recombination frequency*	Comparable values from Vivian & Hopwood (1970)
<i>hisA1</i>	<i>argA1</i>	<i>uraA1</i>	<i>strA1</i>		
39 NF		326 NF		1.6 × 10 ⁻³	1.6 × 10 ⁻²
321 UF		312 UF		10 ⁻⁶	2.9 × 10 ⁻⁴
321 UF		118-4 IF		2 × 10 ⁻⁶	3.9 × 10 ⁻⁴
44 IF		312 UF		7 × 10 ⁻⁶	
39 NF		118-4 IF		2.2 × 10 ⁻⁴	4.0 × 10 ⁻²
				10.0 × 10 ⁻⁴	
44 IF		326 NF		2.3 × 10 ⁻⁴	

* Ratio of selected *ura*⁺*his*⁺ recombinants to minority parental type.

A range of strains with properties intermediate between those of NF and UF strains may be detected in the stock cultures, as well as among the products of crosses NF × UF (Sermonti *et al.* 1971). They have been designated IF (Sermonti *et al.* 1971) by analogy with the IF strains described by Vivian & Hopwood (1970).

To deal with a uniform class of variants, clearly distinguishable both from the NF and the UF types, we have used in this work only those fertility variants which showed the lowest donor ability (with UF testers) together with a very low recipient ability (with NF testers). These correspond best to the description of the IF (initial fertility) strains made by Vivian & Hopwood (1970). IF × UF crosses produce very few recombinants – only a little more than UF × UF crosses, according to Vivian & Hopwood (1970) – while IF × NF crosses may produce recombinants in excess of 10⁻³, being rather more fertile than NF × NF crosses.

IF strains therefore behave approximately as UF in crosses with UF and as NF in crosses with NF.

Although the recombination frequencies between pairs of strains of different fertility type reported by Vivian & Hopwood (1970) are regularly higher than those observed by us, the general picture appears fairly comparable, and the observed discrepancies may well be due to differences in the culture media.

(ii) *Transfer of fertility factor independently from recombination*

As mentioned above, the recombination frequency in mixed cultures of IF × UF strains is very low in our experimental conditions. It is in this type of mixed culture that the transfer of the fertility factor independently from the chromosome markers has been satisfactorily demonstrated. From the spores of mixed culture, parental clones with the UF standard markers were isolated and tested for fertility.

Table 3. *Infectious transfer of a fertility factor in IF × UF crosses*

Parents		Fertility type of the ex-recipient		
Donor IF (code no.)	Recipient UF (code no.)	Total tested (no.)	IF (no.)	Converted (%)
44	316	206	49	24.5
44	316	299	166	55.3
44	312	74	35	47.3
44	312	173	135	78.0

The IF parental genotypes recovered from mixed cultures (about 400 tested) were unaffected.

Table 4. *Loss of recipient ability in formerly UF strains after contact with IF donors*

(Recombination frequency in crosses between an NF indicator and some 'converted' recipients (IF^c). Crosses: IF^c 316 *hisD3 pheA1 strA1* × 39 *hisA1*. Selective medium: MM + phenylalanine.)

IF ^c strain (code no.)	IF ^c strain (no.)*	NF parent (no.)*	Recombinants	
			(no.)*	(× 10 ⁻⁴ minority parent)†
1	75.4 × 10 ⁴	149.8 × 10 ⁴	86.2	1.13
2	14.0 × 10 ⁶	20.0 × 10 ⁶	335.0	0.22
3	30.0 × 10 ⁶	120.8 × 10 ⁶	339.7	0.12
4	45.7 × 10 ⁵	160.0 × 10 ⁵	72.0	0.15
5	5.0 × 10 ⁵	39.0 × 10 ⁵	33.0	0.66
Control (316 UF)	209.0 × 10	400.0 × 10	325.0	1600
Control (316 UF)	234.0 × 10	300.0 × 10	52.0	220

* Average count of three to five dishes (multiplied by the dilution factor).

† Only *hisD3+hisA1+* recombinants were scored.

While about 10⁻⁶ of the cells of the recipient parent appear to have received donor chromosomal markers, the rate of conversion of UF to IF ranged between 24 and 78 % (Table 3). The change from UF to IF is clear-cut, when an NF strains is used as tester. The recipient ability decreased 300 to 1000 times after the change of fertility type so that no error of classification is possible (Table 4). It has also been shown that a UF strain, converted by 'infection' to the IF condition, is in its turn

able to convert another UF strain to IF. Thus the factor appears to be serially transmittable from strain to strain.

(iii) *Effect of acridine orange (AO) on the fertility factor*

(a) *Conversion from IF to UF after treatment by AO*

Growing F⁺ cultures of *Escherichia coli* K 12 are converted to F⁻ in the presence of AO (Hirota, 1960). The same effect has not been observed with FP⁺ of *Pseudomonas aeruginosa* (Stanisich & Holloway, 1969).

Table 5. *Effect of acridine orange (AO) on fertility and transfer of the fertility factor in NF × UF crosses**

Parents		Recombinant frequency		Recipients converted to IF or NF	
Donor NF (code no.)	Recipient UF (code no.)	With AO (%)	Without AO (%)	With AO (%)	Without AO (%)
80	316	49	46	43	55
39	312	29	35	57	94
39	316	59	67	80	71
	Pooled	45.7	49.3	62.7	70.9

* Spores of the two parents were mixed on complete medium supplemented with AO (20 µg/ml) or unsupplemented. After 3–4 days growth the spores of the mixed culture were plated on media selective for two alleles in repulsion (in the three crosses, respectively: *uraA*⁺ and *pheA*⁺, *uraA*⁺ and *hisA*⁺, *strA* and *pheA*⁺) or for the recipient phenotype, as well as on complete medium as control for estimation of recombinant frequency. The recipient phenotypes were scored for loss of high recipient ability in crosses with an NF tester. 120 or more recipients were tested in each experiment.

An effect was evident after plating spores of an IF strain of *S. coelicolor* (118–4) on complete agar medium containing 20 µg/ml AO. The plating efficiency was not affected, but colony growth was strongly inhibited and sporulation was prevented. The grown microcolonies were transferred to master plates of complete medium, where full growth and sporulation was resumed, and they were then tested for fertility against an NF tester. In four experiments about 5% of the microcolonies (4/164, 5/108, 31/503 and 14/226) had clearly achieved the UF condition. No UF variant was obtained from samples of untreated colonies (0/49 and 0/57). Samples of the strain showing the UF response in the spot test were isolated and tested against the NF tester. They gave recombination rates (as percentage of the minority parental type) ranging between 20 and 60%, as compared with 0.10–0.20% for the untreated strain.

(b) *Inhibition of fertility factor transfer by AO*

The most striking effect of AO was observed when IF × UF crosses were carried out on complete media supplemented by AO (20 µg/ml). While in the absence of the drug the fertility factor was transferred to 20–80% of the ex-recipient clones

(Table 3), the transfer was completely prevented in the presence of AO. No converted IF strains were detected among 270 colonies of the UF genotypes (strains 312 or 316) derived from spores of mixed cultures with an IF donor (strain 44) incubated 4 days. Thus the fertility factor appeared much more susceptible to the action of AO during transfer than during reproduction in growing cells. Gene recombination also appeared to be prevented. No recombinant was detected from tens of millions of plating units derived from IF \times UF mixed cultures grown in the presence of AO and plated on selective media.

When NF \times UF crosses were performed in the presence of AO, in exactly the same conditions that prevent transfer of the fertility factor in UF \times IF crosses, no inhibition was observed, either in the recombination rate or in the conversion of the recipient conjugants to donor types (Table 5). The fertility factor is thus sensitive to AO action when moving from IF strains, but completely insensitive when being transferred from NF strains.

4. DISCUSSION

The present results, besides adding new features differentiating the NF (high-frequency donor) and the IF (low-frequency donor) strains of *Streptomyces coelicolor* A3 (2), permit some hypotheses to be put forward on the structural nature of the differences between them. Two alternative hypotheses may be considered: either the fertility factor is in two different *allelic* states in the two types of strain, or it is merely the same factor in different topological conditions. Many observations point to the second alternative. They may be listed as follows:

(i) The donor ability is lost by the IF strains after u.v. or X-ray treatment, but it is retained by similarly treated NF strains (Vivian & Hopwood, 1970).

(ii) Loss of activity in the IF strains is caused by agents effective as phage inducers (u.v. or X-rays) but not by strictly mutagenic agents (NTG) (Vivian & Hopwood, 1970).

(iii) Acridine orange, which is able to remove the F factor from F⁺ (but not from Hfr) strains of *Escherichia coli* (Hirota, 1960), is also, although moderately, effective in abolishing the donor ability of IF (but not of NF) strains of *S. coelicolor*.

(iv) Donor ability is transferred, by cell contact, from IF to UF strains, without concomitant transfer of standard markers.

(v) Donor ability transfer is prevented in the presence of acridine orange, which does not affect donor ability transfer in NF \times UF crosses.

On the first hypothesis the UF condition would be explained as a third allelic state of the fertility factor. The 'topological' hypothesis would require the assumption of the loss of the factor on the origin of the UF strains. All the five listed observations fit the latter assumption, especially (iv) and (v) which clearly point to a 'disinfection' and a 'reinfection' by the factor.

The picture is immediately suggested of a sex factor linked to the chromosome (at the 9 o'clock position) in the NF strains, existing in a free state in the IF strains, and absent from the UF strains. This picture strictly recalls the situation of the F factor in Hfr, F⁺ and F⁻ strains of *E. coli* respectively (Hayes, 1968) with the

main differences of an optional location of the factor in *E. coli* versus a single location of the factor in *S. coelicolor*, and of a leading role of the *E. coli* factor in conjugation versus a possible zygote promoting role of the *S. coelicolor* factor.

The suggested picture is in full agreement with the observation by Vivian & Hopwood (1970) that in NF × UF crosses there is obligate inheritance of the 9 o'clock region of the NF genome by all recombinants, while this is not true in NF × IF crosses. This is to be expected if the fertility factor is assumed to be absent in the UF strain, and therefore needs to be provided by the incoming chromosome. In NF × IF crosses the factor is assumed to be present in the strain acting as recipient (IF) and can support zygote formation even by an incoming fragment not carrying the fertility factor. The hypothesis put forward would assign to the fertility factor of *S. coelicolor* the role of a 'plasmid' or, more exactly, of an 'episome'. The authors are, however, still reluctant to introduce a new nomenclature to designate the fertility types of *S. coelicolor* and prefer to retain the conventional designations (NF, IF, UF) until definite and unquestionable evidence is provided on the actual nature of the fertility factor.

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ADDENDUM

The results of this paper were originally presented by two of us (Sermonti-Spada and Sermonti) at the First Symposium on 'Genetics of Industrial Microorganisms' (Prague, August 1970). Just before the present paper was sent to the press a paper by A. Vivian appeared in the *Journal of General Microbiology* (**69**, 353–364, 1971) showing 'The plasmid involvement in the interconversion of UF and IF strains'. Vivian's conclusion, independently obtained, was based on the observed conversion of the UF parent to IF in IF × UF crosses.