Genetic Control of Fertility in *Streptomyces coelicolor* A3(2): Plasmid Involvement in the Interconversion of UF and IF Strains

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SUMMARY

IF (Initial Fertility) strains of *Streptomyces coelicolor* A3(2) are shown to harbour a plasmid (SCP 1). Loss of SCP 1 results in a change of fertility from IF to the previously described UF (Ultra-Fertile) type. Although UF × IF crosses yield recombinants for chromosomal markers with a frequency of the order of 10^{-5} of the total progeny, virtually all the progeny carrying the markers of the UF parent are converted to IF, by reinfection with SCP 1. Efficient conversion of UF to IF in such crosses is associated with the development of aerial mycelium. It is inferred that SCP 1 is responsible for the production of a diffusible substance by IF strains, which prevents the development of aerial mycelium by UF strains.

INTRODUCTION

Wild-type A3(2) of *Streptomyces coelicolor* is of the Initial Fertility (IF) type (Vivian & Hopwood, 1970). IF strains were found to give rise to Ultra-Fertile (UF) strains (Hopwood, Harold, Vivian & Ferguson, 1968, 1969) spontaneously with an appreciable frequency; from 0.03 to 0.4% of the colonies produced by plating an IF culture are UF, the variation presumably reflecting clonal fluctuations. The frequency of UF production from IF was increased up to tenfold by ultraviolet irradiation and by X-rays, but the chemical mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine had no significant effect (Vivian & Hopwood, 1970). These results implied that the change from IF to UF was unlikely to be due to a simple mutation.

Evidence will be presented in this paper that the change from IF to UF involves the loss of a plasmid, and that this change can be reversed by reinfection of the UF strain with the plasmid, by contact with an IF strain. Further experiments reported here indicate an interaction of the IF and UF fertility types in a mixed culture in relation to the morphological differentiation of the culture.

METHODS

General. Complete (CM) and minimal (MM) media and standard cultural techniques were those described by Hopwood (1967), except that all crosses were performed on CM in glass Petri dishes. For this purpose the glass lid was replaced by an aluminium lid containing a sheet of sterile filter paper. This arrangement was found to favour good growth and better sporulation than the plastic dishes used for all other purposes.

Strains. The strains, which are all mutational and recombinational derivatives of *Streptomyces coelicolor* A3(2) (Hopwood, 1959), are listed in Table 1; some have been described in previous studies of the fertility system (Hopwood *et al.* 1969; Vivian & Hopwood, 1970) and others were produced during the present work.

Ultraviolet irradiation. This was carried out as described by Harold & Hopwood (1970).

Testing of fertility by 'plate crossing'. This was done by a modification of the indirect

selection procedure of Sermonti & Casciano (1963) as described by Hopwood *et al.* (1969) and Vivian & Hopwood (1970).

Genetic analysis. Haploid recombinant selection was done in the way described by Hopwood (1967).

RESULTS

The nature of UF × IF crosses. Mixed cultures of UF and IF strains yield selected recombinants in respect of the usual, chromosomal, markers with a frequency of the order of 10^{-5} of the total spores harvested from the mixed culture (Vivian & Hopwood, 1970). For example, in the cross in Table 2, a selected class of recombinants had a frequency of 2.1×10^{-5} ; approximately 33 % of the spores had the markers of the UF parent and 67 % those of the IF parent. However, when progeny carrying the markers of the UF parent were tested for fertility type, almost all were found to be of the IF type. Since the change of fertility from UF to IF for the parentally marked (*hisA1 uraA1 strA1*) class of segregants occurred with a frequency very much (10,000 times) higher than the frequency of recombinants for chromosomal markers, the factor responsible for the difference in fertility between UF and IF strains must have been inherited independently of the chromosome.

The strains derived from UF strains by contact with IF strains as just described behaved in every way like typical IF strains. In particular they gave rise to UF strains spontaneously, or, after ultraviolet irradiation, at the normal frequencies (Vivian & Hopwood, 1970) (Table 3). They also converted UF strains to IF as efficiently as did type IF strains.

Conversion of UF to IF in mixed culture in relation to colonial differentiation. The growth and development of *Streptomyces coelicolor* is complex for a prokaryote (Hopwood, 1960; Wildermuth, 1970; Wildermuth & Hopwood, 1970). There are three main stages in the development of a colony. Substrate mycelium first develops at, and just below, the agar surface giving a culture which is flat and shiny in appearance. Later aerial mycelium grows upwards from the substrate mycelium and is recognized by its 'hairy', white appearance. Finally spores are formed by septation of the aerial mycelium and the culture surface then becomes grey (Hopwood, Wildermuth & Palmer, 1970). The time scale of development varies with growth conditions (Wildermuth, 1970); in the present experiments aerial mycelium began to appear at about 24 h. and increased up to about 50 h., after which spore production gradually increased, being clearly visible to the naked eye at 72 h.

Replicate crosses of strain 1190 *hisA1 uraA1 strA1* UF with strain 12 *pheA1* IF were incu-

Table 1. *Characteristics of strains*

Strain	Relevant phenotype						†Fertility
	<i>argA1</i>	<i>proA1</i>	<i>hisA1</i>	<i>uraA1</i>	<i>pheA1</i>	<i>strA1</i>	
A3(2)*	+	+	+	+	+	S	IF
12	+	+	+	+	—	S	IF
1098	+	+	+	+	—	S	UF
1141	+	+	+	+	—	S	NF
104	+	+	—	—	+	R	IF
1190	+	+	—	—	+	R	UF
A506	+	+	—	—	+	R	IF
A303	+	+	—	—	+	R	NF
A427	+	+	—	+	+	S	IF
A450	+	+	—	+	+	S	UF
1100	—	—	+	+	—	S	NF

* The wild-type.

† IF = Initial Fertility, NF = Normal Fertility, UF = Ultra-fertile.

Table 2. Results of crossing strains 1190 *hisAI uraAI strAI* UF and 12 *pheAI* IF
The cross was harvested at 72 h.

No. of plating units of each genotype				Fertility of a sample of the <i>hisAI uraAI strAI</i> output
Input		Output		
<i>hisAI uraAI strAI</i> UF	<i>pheAI</i> IF	Parentals	Recombinants	
		<i>hisAI uraAI strAI</i>	<i>pheAI</i>	
0.9×10^6	1.5×10^6	12.5×10^6	22.2×10^6	
		<i>hisAI</i> ⁺ <i>strAI</i>		
		715		
		2.1×10^{-5}		
		I		311

Table 3. Isolation of UF variants after ultraviolet irradiation (u.v.)

The tester strain was 1141 for strain A506 and 1100 for strain 104.

Strain	Treatment	Survival (%)	No. of colonies examined		% UF	
			Total	UF	Total	Induced
A506†	Control	100	2172	19	87	—
	u.v.	0.5	1096	26	2.37	1.50
104*	Control	100	5309	22	0.41	—
	u.v.	0.3	1219	18	1.48	1.07

* Data from Vivian & Hopwood (1970).
† Derived by conversion of strain 1190.

Table 4. Results of a cross between strains 1190 *hisA1 uraA1 strA1* UF and 12 *pheA1* IF:
the effect of harvesting at different times of incubation

The fertility of the *hisA1 uraA1 strA1* progeny was tested with strain 1141.

Hours incubation before harvesting	Culture morphology*			Composition of harvested suspension*			Numbers of progeny		Numbers of each fertility type amongst <i>hisAI uraAI strAI</i>		% IF
	S A Sp			S A Sp			Minority parent	Recombinants <i>hisAI⁺ strAI</i>	progeny		
	S	A	Sp	S	A	Sp			UF	IF	
12	+	-	-	+	-	-	2.4×10^4	0	48	0	0
18	+	-	-	+	-	-	6.2×10^5	0	535	8	2
24	+	±	-	+	±	-	8.3×10^5	3	342	107	24
42(A)†	+	+	-	-	+	-	3.9×10^6	56	2	121	98
42(S)†	+	+	-	+	±	-	4.9×10^5	1	24	172	88
76	+	+	+	-	±	+	5.0×10^6	93	0	47	100

+ = present; ± = small amount present; - = absent. Culture morphology was judged by the appearance of the cultures to the naked eye. Harvested suspensions were examined by phase-contrast microscopy; aerial and substrate mycelium could not be distinguished by this examination, but spores could be recognized unambiguously.

* S = substrate mycelium; A = aerial mycelium; Sp = spores.

† Aerial mycelium was harvested first, the culture washed with water, then substrate mycelium was harvested.

bated for varying time intervals before harvesting (Table 4). At each time of harvest, the suspensions were plated on media selecting each of the two parental marker combinations, and on a medium selecting *hisA1*⁺ *strA1* recombinants. The fertility of a sample of *hisA1 uraA1 strA1* progeny was also determined.

In crosses harvested at 12 and 18 h. the great majority of the *hisA1 uraA1 strA1* progeny remained unchanged in fertility type: that is they were UF. These crosses had not developed aerial mycelium. At 24 h. when aerial mycelium had begun to appear, 24 % of the *hisA1 uraA1 strA1* plating units gave rise to IF colonies. At 42 h. an attempt was made to analyse substrate and aerial mycelium separately. The aerial mycelium was harvested by gentle scraping and plated, and the substrate mycelium was then harvested, after thoroughly washing the previously scraped culture surface. From the aerial mycelium only 2 % of the *hisA1 uraA1 strA1* plating units gave UF colonies, whereas from the substrate mycelium 12 % were UF. It should be emphasized that this experiment probably underestimated the difference between the two stages of development for two reasons. First, it was more difficult to break up the substrate mycelium, so that the plating units were larger than from the aerial mycelium and presumably any IF unit present in a clump of otherwise UF mycelium rendered the resulting colony IF. Results described later tend to support this notion. Secondly, it is unlikely that all the aerial fragments were removed before the substrate mycelium was harvested since they tend to be trapped between the substrate hyphae.

At 76 h. all the *hisA1 uraA1 strA1* colonies were IF. These results suggested that conversion of UF to IF was associated with the production of aerial mycelium, and that the underlying substrate mycelium of the UF parent probably remained at least partly (if not largely) UF.

Addition of water to the Petri dish containing a developing culture, still at the substrate mycelial stage, prevented the development of aerial mycelium although vegetative growth continued. When the water was removed aerial mycelium appeared. In this way it was possible to separate the effects of culture age and colonial differentiation.

Table 5(a) shows the results of an experiment with strains 1190 UF and 12 IF. At 42 h. in the absence of water, aerial mycelium was produced and nearly all the *hisA1 uraA1 strA1* progeny were converted to IF, confirming the results of the previous experiments. However, when water was added at 17 h., when only substrate mycelium was visible, only 3 % of the *hisA1 uraA1 strA1* progeny harvested at 42 h. were IF. Removal of the water at 42 h. and reincubation was followed by aerial mycelium formation at about 90 h. At this time virtually all the *hisA1 uraA1 strA1* progeny were IF.

The experiment was repeated to see whether addition of water at times later than 17 h. could determine a time at which the culture might have become 'committed' to aerial mycelium formation and/or to conversion of fertility type. All the crosses were harvested at 42 h. after the addition of water at times varying from 17 to 28 h. Even when water was added as early as 17 h. a small proportion (2 %) of IF colonies was produced (Table 5b). As the time allowed for growth before the addition of water was lengthened so the proportion of IF progeny amongst the *hisA1 uraA1 strA1* class increased up to 30 % at 28 h., when the culture was beginning to show obvious signs of aerial mycelium.

These results, while not defining a precise time of 'commitment' to IF conversion, clearly establish an association of conversion with aerial mycelium initiation, rather than with the absolute age of the mixed culture.

The effect of varying the input ratio of the two parental strains on the conversion of UF to IF. Experiments were done to see how much the proportion of IF in a mixed culture with UF

Table 5. *The effect of adding water to a UF × IF cross at different times to prevent aerial mycelium development: results of crosses between strains 1190 hisAI uraAI strAI UF and 12 pheAI IF*

In (a) the input ratio of strains 1190:12 = 1:7 and in (b) 1190:12 = 3:2. The fertility of the *hisAI uraAI strAI* progeny was tested with strain 1141.

Time of harvest (h.)	Time of addition or removal of water (h.)		Numbers of progeny		Numbers of each fertility type amongst <i>hisAI uraAI strAI</i> progeny		% IF
	Added	Removed	minority parent	recombinants <i>hisAI⁺ strAI</i>	UF	IF	
(a)	42	—	2.4×10^4	7	1	221	99
	42	—	4.2×10^4	0	435	12	3
	90	42	4.1×10^4	589	4	313	99
(b)	42	—	3.0×10^5	53	4	131	97
	42	—	*	*	633	10	2
	42	—	1.6×10^7	46	619	47	7
	42	—	2.2×10^7	96	370	66	15
	28†	—	2.4×10^7	95	316	136	30
	42	—					

* Lost through contamination.

† Portion of culture showing aerial mycelium to the naked eye at time of addition of water.

Table 6. The effect on fertility conversion of input ratio of the two parental strains in a UF × IF cross.

(a) Results for strains 1190 *hisAI uraAI strAI* UF and 12 *pheAI* IF.
(b) Results for strains 1098 *pheAI* UF and 104 *hisAI uraAI strAI* IF.

Time of harvest (h.)	Approximate ratio <i>hisAI uraAI strAI:pheAI</i>		Number of input plating units		Number of <i>hisAI⁺ strAI</i> recombinants per minority parent in output	Numbers of each fertility type*		% IF
	Input	Output	IF parent	total		UF	IF	
(a)								
72	0.7:1	4:1	2.2 × 10 ⁶	3.6 × 10 ⁶	3.4 × 10 ⁻⁴	1	379	99
72	7:1	12:1	4.4 × 10 ⁵	3.2 × 10 ⁶	2.6 × 10 ⁻⁴	1	215	99
72	700:1	23:1	4.4 × 10 ³	2.8 × 10 ⁶	8.1 × 10 ⁻⁵	95	13	12
96	70:1	100:1	4.4 × 10 ⁴	2.8 × 10 ⁶	3.8 × 10 ⁻⁴	2	411	99
96	700:1	50:1	4.4 × 10 ³	2.8 × 10 ⁶	1.2 × 10 ⁻⁴	1	238	99
(b)								
72	1:0.25	1:9	5.8 × 10 ⁶	7.0 × 10 ⁶	2.2 × 10 ⁻⁴	2	226	99
96	1:25	1:7	1.2 × 10 ⁵	2.5 × 10 ⁶	2.8 × 10 ⁻⁴	12	870	99
96	1:250	1:30	1.2 × 10 ⁴	2.4 × 10 ⁶	1.8 × 10 ⁻⁴	374	209	36
96	1:2500	1:15	1.2 × 10 ³	2.4 × 10 ⁶	4.2 × 10 ⁻⁴	324	31	9

* For (a) the *hisAI uraAI strAI* progeny tested with strain 1141, and for (b) the *pheAI* progeny tested with strain A303.

could be reduced, while still allowing efficient conversion of the UF parent to IF. Table 6(a) shows results obtained for strains 1190 *hisA1 uraA1 strA1* UF $\times$ 12 *pheA1* IF and Table 6(b) for strains 1098 *pheA1* UF $\times$ 104 *hisA1 uraA1 strA1* IF. The purpose of crosses involving the same fertility types in reciprocal coupling with the markers was to test for gross distortion of output ratios by marker effects; the results suggest that the markers did not greatly affect the output ratios of the crosses. In both crosses, for approximately equal input ratios the UF marker combination tended to increase relative to the IF combination in the output, whereas for the most unequal input ratios there was a strong tendency to reduce the excess input of the UF marker combination.

The results indicate that at input ratios of UF:IF up to about 25:1 in cross (b) and even higher in cross (a), conversion of the UF parental class was close to 100%. Table 6(a) (lines 3 and 5) indicate that a majority of UF at 72 h. became a majority of IF when incubated for a further 24 h., even when the input ratio of UF to IF was as high as 700:1.

It should be emphasized that the absolute age of the various crosses was a poor indicator of their physiological age. Crosses in which the IF parent was in a small minority took longer to reach a state of aerial development comparable with the cross involving equal inputs of parental types. This was probably not due to variation in the total number of spores used to make the crosses because efforts were made to minimize such differences by adjusting the total concentrations of the mixed suspensions. The total spore input in the crosses never varied more than threefold (column 5, Table 6).

A possible conclusion from the results of Table 6 is that UF is at a disadvantage in producing aerial mycelium when in mixed culture with IF, since a small minority of IF effectively 'suppressed' aerial mycelium production in a large majority of UF input. This possibility was tested directly in experiments described in the next section. Such an effect could also explain the very efficient conversion of UF to IF in the aerial mycelium of a mixed culture, but not in the substrate mycelium, described earlier; 'converted' (IF) substrate mycelium would be able to proceed to aerial mycelium development, while unconverted (UF) would not.

The differential ability of UF and IF components in a mixed culture to produce aerial mycelium. An observation suggesting that UF mycelium may be prevented by IF from initiating the aerial phase was the following. When a plate of colonies, some IF and some UF, was crossed by replica plating to a lawn of a UF tester strain, the two types of colony could be distinguished by their effect upon the growth of the UF tester strain (Fig. 1): the IF colonies produced a halo in the UF background where no aerial mycelium developed, whereas the UF colonies did not. The fertility of the two types of colony, as described previously (Vivian & Hopwood, 1970), was similarly low with the UF tester, but markedly different with an NF (Normal Fertility) tester (Fig. 1c).

Figure 2 shows the results of a more direct investigation of this phenomenon. UF or IF strains carrying the marker *hisA1* were grown in the centre of a Petri dish and replicated to a lawn of either a UF or an IF strain carrying the markers *hisA1 uraA1 strA1*. Thus, at the centre of each dish there was a mixed culture of IF $\times$ IF, IF $\times$ UF or UF $\times$ UF surrounded respectively by IF, UF or UF growth.

The mixed IF on UF culture (dish 2) is surrounded by a zone where the UF culture did not produce aerial mycelium. Absence of this zone around homologous UF on UF (dish 1) and IF on IF (dish 3) crosses indicates that it was not due to a starvation effect caused by the increased inoculum in the mixed region, nor to an effect of the markers in the strains.

Petri dishes 4 to 9 in Fig. 2 are replicas of their respective crosses (dishes 1 to 3) on media which allow distinction of the two parental marker combinations. Clearly there was no migration of the IF markers beyond the mixed zone.

Fig. 1 (a) *hisA1 uraA1 strA1* progeny from a mixed culture of 1190 *hisA1 uraA1 strA1* UF and 12 *pheA1* IF. (b) Plate cross on CM prepared by replicating plate (a) to a lawn of tester strain 1098 *pheA1* UF. IF colonies on the UF background are surrounded by a dark zone where aerial mycelium development of the UF tester strain has been prevented; UF colonies on the same plate are not. The UF and IF colonies are distinguished by plate (c). The colonies shown in (a) were plate crossed to tester strain 1141 *pheA1* NF. This plate cross (not shown) was then replicated to the plate of selective medium in (c) which distinguishes the colonies on the basis of their fertility with the NF tester. UF colonies give many recombinants; IF colonies give few or no recombinants under these conditions.

Petri dishes 1 to 3 were also replicated to a suitably marked NF tester strain, 1141 *pheA1* which allowed the fertility of both the parental types to be tested. The plate crosses so produced (which are not shown in Fig. 2) were then replicated to a medium selecting *hisA1⁺ pheA1⁺* recombinants. The result is shown in Fig. 2, dishes 10 to 12. As expected, UF on UF gave a high frequency of recombinants over the whole plate (dish 10) whereas IF on IF gave a low level over the whole plate (dish 12). The cross of IF on UF (dish 11) showed an inner zone where the mixed IF + UF inoculum gave a low level of recombination consistent with its

having become entirely IF by conversion of the UF parent. Outside of this area, the region devoid of aerial mycelium on the original plate (dish 2) did not replicate and hence gave no recombinants. Beyond this zone the fertility of the UF parent remained high, indicating that there was no migration of IF fertility outwards through the UF parent.

A small piece of substrate mycelium from the region devoid of aerial mycelium in dish 2 was picked to a fresh plate of CM and streaked out. Colonies of normal appearance

developed with aerial mycelium and spores. These were tested for fertility and genotype and found to be *hisA1 uraA1 strA1* UF, showing that the UF strain in the region where aerial mycelium development had been inhibited, had not undergone a permanent change.

DISCUSSION

The frequency of UF production from IF strains is higher than that expected for a chromosomal mutation (Hopwood *et al.* 1968, 1969; Vivian & Hopwood, 1970) but is compatible with its being due to loss of a plasmid. The finding that IF strains can 'convert' UF strains to IF with a frequency almost 10,000-fold higher than the frequency of inheritance of chromosomal markers, and that such 'converted' strains are as stable as type IF strains, clearly demonstrates that the change from UF to IF involves the acquisition of an extra-chromosomal element: by definition, a plasmid. It is proposed to call the plasmid SCP 1 (*Streptomyces coelicolor* plasmid 1).

It seems that SCP 1 is responsible either directly or indirectly for the production of a diffusible substance which prevents or reduces the initiation of aerial mycelium by cultures lacking SCP 1. Thus IF itself, or an intimate IF + UF mixture, prevents the development of UF aerial mycelium. It is possible that production of this developmental inhibitor has to be induced by the presence of a strain lacking SCP 1.

In a UF × IF cross, the IF strain, which harbours SCP 1, is able to produce aerial mycelium and thereafter spores, whereas any part of the culture which lacks the plasmid (and so remains UF) is prevented by the IF from developing aerial mycelium, and hence spores. It is possible that the plasmid is transferred to a minority of the UF substrate mycelium, as indicated by the results of harvesting a UF × IF cross at times close to aerial mycelium initiation (18 h.) (Table 4). The dramatic increase in the proportion of UF converted to IF is clearly associated with the development of aerial mycelium and it is not necessary to postulate a progressive conversion of the underlying substrate mycelium. The end result, as judged by the analysis of *spores* from the mixed culture, is almost total conversion of the UF parent to IF. The results obtained in Table 5 discount the possibility that age of the culture *per se* is responsible for the efficient conversion of UF to IF.

Differences previously observed between UF and IF strains centred on their respective behaviours when crossed with NF strains. The most obvious difference was that UF strains were more fertile (indeed 'ultra-fertile') with NF than were IF strains: the frequency of

Fig. 2. Suppression by IF of aerial mycelium production by a UF strain: for full explanation see text.

1 to 3. Plate crosses on CM. (1) A central patch of A450 *hisA1* UF was replica plated on to a lawn of 1190 *hisA1 uraA1 strA1* UF. (2) As (1) but A427 *hisA1* IF on a lawn of 1190 *hisA1 uraA1 strA1* UF. As (1) but A427 *hisA1* IF on a lawn of 104 *hisA1 uraA1 strA1* IF.

4 to 6. Replicas from plate crosses 1 to 3 respectively on medium selecting for *hisA1* parental phenotype.

7 to 9. Replicas from plate crosses 1 to 3 respectively on medium selecting for *hisA1 uraA1 strA1* parental phenotype.

10 to 12. Plate crosses 1 to 3 were replica plated to further plate crosses (not shown here) with 1141 *pheA1* NF as tester strain. 10 to 12 are the respective replicas from these plate crosses on medium selective for recombinants. (10) The level of recombination with NF is high, confirming that the growth on 1 is all UF. (11) The inner circular area gives few recombinants, showing that it has become uniformly IF. Outside of this is a ring where aerial mycelium development has been prevented; this region failed to replicate and so gave no recombinants. Around this the culture remains UF and gives many recombinants. (12) The level of recombination with NF is low, confirming that the growth on 2 is all IF.

recombinants in a UF $\times$ NF cross reaches 100% (Hopwood *et al.* 1968, 1969), whereas in an IF $\times$ NF cross it is around 10% (Vivian & Hopwood, 1970). The increased fertility of UF $\times$ NF as compared with IF $\times$ NF crosses can be visualized as due to the abolition of asexual reproduction in the former, but not in the latter. It is a reasonable hypothesis that this is due to SCP I harboured by the NF strain. Because of the ultra-fertile nature of UF $\times$ NF crosses it has not been possible to demonstrate conversion of UF by NF independently of the transfer of chromosomal markers. In other words, if NF does harbour SCP I, its inheritance must be associated with that of chromosomal markers since all recombinant progeny from UF $\times$ NF crosses are NF (Hopwood *et al.* 1969).

It should be emphasized that loss or gain of SCP I is not associated with a change from donor to recipient or vice versa, since both IF and UF strains behave as recipients in crosses with NF strains. The difference between donor and recipient behaviour has been located chromosomally by crossing NF and IF strains (Vivian & Hopwood, 1970). Thus, although the implication of a plasmid in the control of sexual reproduction in *Streptomyces coelicolor* is in itself reminiscent of the situation in eubacteria like *Escherichia coli* (Hayes, 1968), there are at present no close analogies between the two systems. On the contrary, the association between a fertility factor and colonial differentiation described in this paper, promises to illuminate problems which are beyond the scope of studies with the simple eubacteria.

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