Polarity of Gene Transfer in *Caulobacter*

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INTRODUCTION

Jollick & Schervish (1972) presented evidence for genetic recombination in *Caulobacter*. Both members of the mating pairs were shown to be required for recombination, since lysates of either parent of a positive cross could not effect recombination when mixed with intact cells of the other parent. This observation supports similar findings by Shapiro, Agabian-Keshishian & Bendis (1971), and leads to the conclusion that genetic recombination in this genus occurs by conjugation.

The unidirectional nature of gene transfer as described in *Escherichia coli* K12 by Hayes (1952), implied sexuality in bacteria; this was later demonstrated in *E. coli* by Hayes (1953), Lederberg, Cavalli & Lederberg (1952), and other investigators (Jacob & Wollman, 1961), with the discovery and characterization of F factors. Similar F-like factors have been reported in *Pseudomonas* (Holloway & Jennings, 1958), *Vibrio* (Bhaskaran 1964; Datta *et al.* 1973) and in *Streptomyces* (Hopwood *et al.* 1972). Since each of these mating systems exhibited a polarity of gene transfer, it was reasonable to determine if a similar phenomenon existed in the conjugation system of *Caulobacter*. Experiments similar to those of Hayes, which showed that the viability of only one member of a mating pair is essential to gene transfer, are reported here.

METHODS

Bacterial strains. Procedures for constructing strains have been previously reported (Jollick & Schervish, 1972). Streptomycin-resistant (*str-r*) mutants were selected directly on streptomycin-containing medium. The strains used are listed in Table 1.

Media. Complete medium was the peptone-yeast extract (PYE) medium of Poindexter (1964). Streptomycin- or penicillin (Pen)-resistant mutants were selected on PYE medium supplemented with 200 µg streptomycin sulphate or penicillin/ml, respectively. Minimal medium consisted of (% w/v): glucose, 0.2; (NH₄)₂SO₄, 0.1; K₂HPO₄, 0.05; KH₂PO₄, 0.05; MgSO₄·7H₂O, 0.01; agar, 1.5. Histidine, methionine and arginine were added to minimal medium where appropriate. The concentration of the amino acid supplements was 2.0 µg/ml.

Mating procedures. Control matings were performed by mixing 1.0 ml of an 18 to 24 h PYE broth culture of each partner. The viable titre of each strain was standardized at 1.5×10^8 to 3.0×10^8 colony-forming units/ml before mixing. All mating mixtures were incubated at 30 °C for 2.0 h and then 0.1 ml of each mixture was spread on solid minimal medium (containing appropriate supplements where required). Each individual member of the cross was tested for reversion.

To test the polarity of gene transfer and identify donor and recipient strains, matings were performed as described above, except that streptomycin (200 µg/ml, final concentration)

Table 1. *Caulobacter* strains

Strain designation*	Proposed mating type	Genotype	Source
CB15	Donor	Prototroph	ATCC19089
WS1	Donor	<i>asp-1 str-s</i>	CB15
WS2	Donor	<i>his-1 str-s</i>	CB15
WS3	Donor	<i>asp-2 str-s</i>	CB15
WS4	Donor	<i>his-2 str-s</i>	CB15
WS5	Donor	<i>his-3 str-s</i>	CB15
WS6	Donor	<i>his-4 str-s</i>	CB15
WS9	Donor	<i>his-5 str-s</i>	CB15
WS12	Recipient	<i>met-3 str-s</i>	CB15
WS18	Donor	<i>asp-1 str-r</i>	WS1
WS19	Donor	<i>his-1 str-r</i>	WS2
WS20	Donor	<i>his-3 str-r</i>	WS5
WS25	Donor	<i>ser-1 str-s</i>	CB15
WS26	Donor	<i>ser-1 str-r</i>	WS25
WS28	Donor	<i>trp-2 str-s</i>	CB15
WS30	Recipient	<i>met-3 str-r</i>	WS12

* The strain designation CB15 is the original one of Poindexter (1964), and is retained in this paper; ws designations are those strains isolated by the authors.

was added to the mating mixture and after the mating incubation of 2 h the culture was centrifuged and the pellet resuspended in fresh PYE broth before plating on the appropriate selective medium.

In a variation of the above, the matings between *str-r* and *str-s* strains were performed by our standard mating procedure, then plated on minimal medium and minimal + streptomycin (200 µg/ml).

In all matings the plates were incubated at 30 °C and checked at 5, 7 and 12 days. Recombinant colonies were counted at 12 days.

Matings on solid media. Once donor strains were identified it was important to establish the stability of the donor property during storage. Donors that were stored on complete agar slants were periodically streaked on to complete medium and at least 60 isolated colonies picked and transferred to gridded PYE plates which serve as the donor master plates. The recipient strain was grown in PYE broth, and after 24 h the bacteria were collected by centrifugation and resuspended in distilled water; this suspension was spread uniformly on a minimal plate. The donor master plate was then replica-plated on to the minimal plate spread with the recipient strain. The plates were incubated at 30 °C and examined at 5, 7 and 12 days for prototroph (recombinant) formation.

RESULTS AND DISCUSSION

To show the polarity of gene transfer in bacteria one must demonstrate that each parent in a cross does not play an equivalent role in the formation of recombinants. The experiments described here were suggested by those used by Hayes (1952) to show polarity of transfer in *E. coli*. The basic point is that the viability of one parent (the recipient) is necessary for expression of the recombinant genotype whereas the viability of the donor is of no consequence once the act of transfer is accomplished.

Identification of fertile auxotrophic mating strains. We determined from preliminary matings of our auxotrophs that several mating combinations gave consistently high frequencies of recombinant formation and showed very low frequencies of reversion to prototrophy. Auxotroph WS12 was one of our better mating auxotrophs. It produced recombinants with

Table 2. *The effect of streptomycin in the mating mixture on recombinant formation with str-r and str-s mating combinations*

Cross	No. of recombinant colonies		Cross	No. of recombinant colonies	
	No streptomycin	Plus streptomycin		No streptomycin	Plus streptomycin
WS18 × WS12	120	0	WS1 × WS30	300	12
WS19 × WS12	290	0	WS2 × WS30	210	4
WS20 × WS12	205	0	WS5 × WS30	113	12
WS26 × WS12	201	0	WS25 × WS30	433	17

several other auxotrophs including WS1, WS2, WS3, WS4, WS5, WS6, WS25 and WS28. Recombinant frequencies ranged from 5×10^{-6} to 8×10^{-7} recombinants per recipient cell plated.

Mating in the presence of streptomycin. Following the identification of fertile pairs, we made each partner *str-r* and then mated *str-s* and *str-r* combinations of the known fertile pairs in the presence of streptomycin as described above. The *str-r* strains WS18 (derived from WS1), WS19 (derived from WS2), WS20 (derived from WS5), and WS26 (derived from WS25) produced recombinants when mated with WS12 in matings without streptomycin (Table 2). Likewise, recombinants were produced when WS30 (*str-r* derivative of WS12), was mated with WS1, WS2, WS5 and WS25. When streptomycin was added to the mating mixtures, no recombinants were formed in crosses with WS12 (*str-s*) even though the other partner in the mating was *str-r*. We did note, however, a relatively low frequency of recombination when WS30 (*str-r*) was mated with WS1, WS2, WS5 and WS25 (all *str-s*) in the presence of streptomycin. The number of recombinants formed in these crosses in the presence of streptomycin was reduced to less than 10 % of the control. These data suggest that the viability of WS12, or its *str-r* derivative WS30, was required for recombinant formation, and the viability of WS1, WS2, WS5 or WS25 was not essential. The exposure of the mating partners to streptomycin for the full 2 h of the mating incubation could be responsible for the reduction in the number of recombinants recovered from the WS30 crosses seen in Table 2.

Mating followed by recombinant selection on streptomycin containing minimal medium. Mating mixtures of WS1 × WS12, WS1 × WS30, WS18 × WS12 and WS18 × WS30 were incubated for 2 h then plated on minimal and minimal + streptomycin media. On the minimal medium (control), recombinants were detected in numbers consistent with earlier matings with these strains (Table 3). However, on the streptomycin-containing minimal medium no recombinants could be seen in the WS1 × WS12 cross. In the WS1 × WS30 cross the number of recombinants detected represented 3.50 % of those seen with the control, while in the reciprocal cross WS18 × WS12 the recombinants seen represented only 0.35 % of the control cross. In the crosses where both parents were *str-r* (WS18 × WS30) the number of recombinants equalled 8.50 % of the control cross. The results (Table 3) again showed that the viability of strain WS12 or its *str-r* derivative was essential to recombinant formation while the viability of the other partner was not.

In both types of experiment the presence of streptomycin reduced the number of recombinants formed, even when two streptomycin-resistant parents were mated. This suggests that streptomycin interferes in some way with mating or recombination, or both. However, in each type of experiment it appeared that WS12, or its derivative WS30, must retain viability. We therefore designated WS12 and its derivatives as recipients and WS1 and its *str-r* derivatives as donors.

Test of donor stability. Donor strains WS1, WS6 and WS9 which mated with WS12 and its

Table 3. *The effect of streptomycin in the selective medium on recombinant formation by str-r and strs- mating combinations*

Cross	No. of recombinant colonies on minimal plates	
	No streptomycin	Plus streptomycin
WS1 × WS12	300	0.1
WS1 × WS30	341	12.0
WS18 × WS12	141	0.5
WS18 × WS30	200	17.0

derivatives, were periodically tested for the retention of donor capacity after storage. Strains stored in frozen skim milk showed no detectable loss of the donor property for up to three months. When strain ws9 was stored on solid medium at 4 °C, approximately 50 % of the cells lost the donor property, whereas strains ws1 and ws6 did not lose the donor property under these conditions. The tests of the stability of the donor property during storage indicated that most strains designated as donors do retain the property; we have also shown the same stability with several lyophilized strains.

We conclude that unidirectional gene transfer does occur in conjugating strains of *Caulobacter*.

Note added in proof. In a recent report (Gene transfer in *Caulobacter crescentus*: Polarized inheritance of genetic markers. *Genetics* **80**, 1-11.) Newton and Allebach presented evidence which indicates polarity of gene transfer in *Caulobacter*. Their evidence includes 'the preferential inheritance of unselected markers from the non-fertile parent', which means the majority genotype of their recombinants is that of the 'non-fertile' or recipient strain. Although our methodology, strains and some of our findings differ, we feel that our report and that of Newton and Allebach confirm the existence of donor and recipient cell types in *Caulobacter*.

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