Genetic Recombination in *Caulobacter*

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SUMMARY

Auxotrophic, phage-resistant and streptomycin-resistant mutants of *Caulobacter* were used to demonstrate genetic recombination in this genus. Forty of a possible 170 mating pairs gave recombinant frequencies of from 100 to 1000 times greater than reversion frequencies for the markers used. Stability of the recombinants was demonstrated by successive cloning on both complete and minimal medium. Cell contact is apparently required for recombination since both cell types of a mating pair must be present for prototroph formation.

INTRODUCTION

Caulobacter is a stalked pseudomonad found in aquatic, marine and soil environments. This genus has been characterized by Poindexter (1964), and Schmidt & Stainer (1965) have reported both DNA and RNA containing bacteriophage for *Caulobacter*. Because of its diphasic life cycle this organism has been suggested as a model for the study of cell differentiation. *Caulobacter* exhibits two distinct morphological types, a non-motile stalked cell and a motile cell with no stalk. The motile cell is the immature antecedent to the stalked form. Stalk formation precedes cell division from which two daughter cells, one stalked, the other motile, arise. The stalked cell may divide indefinitely, while the motile cell must first form a stalk before division. The desirability of developing a system of genetic analysis is apparent, if *Caulobacter* is to be used as a model for differentiation studies. Shapiro, Agabian-Keshishian & Bendis (1971) have introduced evidence for genetic recombination in *Caulobacter*. This report substantiates her finding and presents additional information concerning genetic recombination in *Caulobacter*.

METHODS

Organisms. *Caulobacter crescentus* ATCC 19089 was chosen as the wild parent prototroph for derivation of auxotrophic mutants. The strain designation CB15, used by Poindexter (1964), is retained in this paper.

RNA bacteriophage ATCC 19089B₁ (ϕ B₁), was used to select phage-resistant mutants of the auxotrophs.

Growth conditions. Cultures were incubated at 30 °C in all experiments. The complete medium was the Peptone Yeast extract (PYE) medium of Poindexter (1964). The chemically defined (minimal) medium consisted of: glucose, 0.2 %; (NH₄)₂SO₄, 0.1 %; K₂HPO₄, 0.05 %; KH₂PO₄, 0.05 %; MgSO₄·7H₂O, 0.01 %; agar, 1.5 %. Bacteriophage were propagated on the complete medium by the agar layer method of Adams (1959).

Isolation of auxotrophs. A 15 ml PYE broth culture of the parent strain containing

2.0×10^8 colony-forming units (c.f.u.)/ml was centrifuged and resuspended in 10 ml of a fresh, filter-sterilized saline solution of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (NTG), 0.02 %. The suspension was incubated at 30 °C for 20 min. After incubation the cells were sedimented by centrifugation and resuspended in fresh PYE broth. Serial tenfold dilutions of the treated cells were plated on PYE agar. Colonies of survivors of NTG treatment were then picked to complete (PYE) and minimal agar plates. Auxotrophs were identified as those isolates which grew on complete but not on minimal medium. The growth requirements of the auxotrophs were established by streaking the auxotroph on the minimal medium and then applying small filter paper discs saturated with solutions containing six amino acids. The procedure was then repeated using solutions of single amino acids to establish the exact growth requirement.

Isolation of streptomycin- and phage-resistant auxotrophs. Phage-resistant mutants of the auxotrophs were selected by an enrichment technique. Phage-infected broth cultures (MOI 1–10) were serially passed through five 48 h subcultures. A small sample from the fifth passage was streaked on complete agar, the colonies were picked and tested for phage resistance and retention of auxotrophy. Auxotrophs were tested for phage resistance by dropping serial tenfold dilutions of a high titre phage suspension on a PYE overlay seeded with the strain in question. Sensitive strains showed plaque formation in the spot areas. Resistant strains were unaffected by the phage at all dilutions tested.

Streptomycin-resistant mutants were selected by spreading a 24 h PYE broth culture containing 2.5×10^{10} c.f.u./ml on a streptomycin gradient plate. The antibiotic concentration was from 0 to 200 µg/ml. Streptomycin resistance was tested using PYE agar plates with streptomycin added to a final concentration of 100 µg/ml.

Mating experiments. The matings were performed by mixing 1.0 ml of an 18 to 24 h PYE broth culture of each partner. The titre of the individual strains was standardized to 1.5 to 3.0×10^8 c.f.u./ml before mixing. The mixture was incubated at 30 °C for 2.0 h then 0.1 ml of each mixture was spread on minimal agar, incubated and the colonies scored. For controls, each individual strain was also plated in like numbers on minimal agar to check for reversion to prototrophy.

RESULTS AND DISCUSSION

Nineteen independent amino acid-requiring auxotrophs with various combinations of phage and streptomycin resistance were obtained from the wild parent (Table 1). Eight of them were made phage-resistant, and three stable streptomycin-resistant mutants were also acquired.

All mating combinations possible with the nineteen strains were tried. The results of several typical mating experiments are presented in Table 2. Although only thirteen crosses are shown in Table 2, forty of the 170 possible mating pairs consistently produced prototrophs at a frequency of from 100 to 1000 times greater than observed by reversion of the auxotrophs. The recombination frequency for prototrophy in our best system was one recombinant/ 10^5 pairs plated. Shapiro *et al.* (1971) reported a frequency of recombination of 1.5 to 1.7×10^{-6} with their *str-s bio*⁺ and *str-r bio*⁻ strains. These frequencies are lower than the ones we report here. The differences may reflect the different strains or mating technique employed. Shapiro reports a spontaneous reversion rate for her *str-r* to *str-s* of 2×10^{-8} which is very close to that of our CB15 auxotrophs where the highest spontaneous reversion frequency observed was 2.5×10^{-8} .

The stability of the presumed recombinants was tested several ways. Sixty individual recombinant colonies from each positive cross were picked and streaked on both minimal

Table 1. *Genotypes of mutants derived from Caulobacter crescentus* ATCC 19089

Strain designation CB15 (ATCC 19089)	Genotype	
	Prototroph	ϕB_1 -s str-s
A1	<i>asp-1</i>	ϕB_1 -r str-r
A2	<i>his-1</i>	ϕB_1 -r str-r
A3	<i>asp-2</i>	ϕB_1 -s str-s
A4	<i>his-2</i>	ϕB_1 -r str-s
A5	<i>his-3</i>	ϕB_1 -s str-s
A6	<i>his-4</i>	ϕB_1 -s str-s
A7	<i>met-1</i>	ϕB_1 -r str-r
A8	<i>met-2</i>	ϕB_1 -r str-r
A9	<i>his-5</i>	ϕB_1 -s str-s
A10	<i>gly-1</i>	ϕB_1 -r str-s
A11	<i>try-1</i>	ϕB_1 -r str-s
A12	<i>met-3</i>	ϕB_1 -r str-s
A13	<i>his-6</i>	ϕB_1 -s str-s
A21	<i>met-4</i>	ϕB_1 -s str-s
A23	<i>met-5</i>	ϕB_1 -s str-s
A25	<i>ser-1</i>	ϕB_1 -s str-s
A28	<i>try-2</i>	ϕB_1 -s str-s
A39	<i>try-3</i>	ϕB_1 -s str-s
A40	<i>met-6</i>	ϕB_1 -s str-s

Table 2. *Recombinants formed from auxotrophic mating pairs*

Mating pair	Controls*	Recombinations† (prototrophs)	Frequency of prototroph formation by reversion (per 10 ⁵ cells plated)	Frequency of prototroph formation by recombination (per 10 ⁵ mating pairs plated)
A1 × A7	0, 1	1000‡	0.0005	1.00
A1 × A10	0, 2	296	0.001	0.30
A4 × A7	1, 1	1000	0.001	1.00
A4 × A12	1, 1	1000	0.001	1.00
A5 × A7	0, 1	103	0.0005	0.10
A5 × A10	0, 2	45	0.001	0.05
A5 × A12	0, 1	65	0.0005	0.05
A3 × A21	3, 0	1000	0.0015	1.00
A3 × A23	3, 0	1000	0.0015	1.00
A3 × A25	3, 2	301	0.0025	0.30
A3 × A40	3, 1	1000	0.002	1.00
A13 × A25	0, 2	203	0.001	0.20
A13 × A40	0, 1	40	0.0005	0.04

* Average number of colonies appearing on minimal medium when the parent cultures are plated alone.

† Average number of colonies appearing on minimal medium when the mating pair mixtures are plated.

‡ Actual counts ranged from 800 to 1300 colonies in repeated experiments.

and complete media and then five randomly chosen colonies from each were picked and passed at least twice more on minimal. Recombinant colonies were also picked to complete broth, assayed for viable count on both complete and minimal agar then streaked on minimal for three passes. Only two of the crosses tested showed less than 90 % of the colonies picked to be unstable prototrophs. A random check of the stability of the prototrophs formed from various matings is shown in Table 3.

Progeny of several of the crosses were examined for the two unselected markers: phage resistance and streptomycin resistance. Unfortunately some of the mating combinations

Table 3. *Stability of prototrophy in recombinants from auxotrophic mating pairs*

Mating pair	Total no. of presumed recombinants tested	No. of recombinant colonies demonstrating stable prototrophy	Stable recombinants (%)
A1 × A7	60	60	100
A1 × A10	60	60	100
A4 × A7	60	58	97
A4 × A12	52	49	94
A5 × A7	37	34	92
A5 × A10	45	43	95
A5 × A12	60	60	100
A3 × A21	60	35	54
A3 × A23	60	59	99
A3 × A25	60	57	95
A3 × A40	60	58	97
A13 × A25	60	57	95
A13 × A40	40	40	100

Table 4. *Crosses between mating partners with triple marker differences*

Cross	Genotypes of mating partners	Genotypes possible in recombinants	Recombinants of each genotype (%)
A3 × A7	<i>met</i> ⁺ <i>asp</i> -2 <i>φ</i> B ₁ -s <i>str</i> -s	<i>met</i> ⁺ <i>asp</i> ⁺ <i>φ</i> B ₁ -s <i>str</i> -s	96
	×	<i>met</i> ⁺ <i>asp</i> ⁺ <i>φ</i> B ₁ -s <i>str</i> -r	0
	<i>met</i> -I <i>asp</i> ⁺ <i>φ</i> B ₁ -r <i>str</i> -r	<i>met</i> ⁺ <i>asp</i> ⁺ <i>φ</i> B ₁ -r <i>str</i> -s	4
		<i>met</i> ⁺ <i>asp</i> ⁺ <i>φ</i> B ₁ -r <i>str</i> -r	0
A25 × A7	<i>met</i> ⁺ <i>ser</i> -I <i>φ</i> B ₁ -s <i>str</i> -s	<i>met</i> ⁺ <i>ser</i> ⁺ <i>φ</i> B ₁ -s <i>str</i> -s	90
	×	<i>met</i> ⁺ <i>ser</i> ⁺ <i>φ</i> B ₁ -r <i>str</i> -r	0
	<i>met</i> -I <i>ser</i> ⁺ <i>φ</i> B ₁ -r <i>str</i> -r	<i>met</i> ⁺ <i>ser</i> ⁺ <i>φ</i> B ₁ -r <i>str</i> -s	10
		<i>met</i> ⁺ <i>ser</i> ⁺ <i>φ</i> B ₁ -r <i>str</i> -r	0

that would have provided the most information did not produce prototrophs. However, several positive crosses occurred between partners with at least two different markers: one the auxotrophic marker and either phage or streptomycin resistance. In two of the positive crosses, auxotrophs A7 × A25 and A3 × A7, the parents had three different markers: Table 4 illustrates the genotypes of the mating pairs and the expected and observed progeny from each cross.

Since we were selecting for prototrophy all recombinants would be *met*⁺ *asp*⁺ in the first cross and *met*⁺ *ser*⁺ in the second. The other markers could theoretically be found in any of the combinations shown. However, none of the recombinants we tested were streptomycin resistant. We have insufficient data to speculate as to the reason for this observation. The mechanism of genetic transfer is presumed to be conjugation since preliminary tests indicate that both cell types must be present in the mating mixtures and supernatants of one member of a positive pair when mixed with viable cells of its partner do not produce prototrophs. This observation supports Shapiro's contention that cell contact is required for genetic exchange.

Many aspects of the genetics of *Caulobacter* remain to be explored. However, the stability of the prototrophs derived from the auxotrophic mating pairs, and the indication of re-shuffling of the phage and antibiotic resistant markers seen in the examination for unselected characteristics, provide evidence for genetic recombination in *Caulobacter*.

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REFERENCES

- ADAMS, M. H. (1959). *Bacteriophages*. New York: Interscience Publishers.
- POINDEXTER, J. S. (1964). Biological properties and classification of the *Caulobacter* group. *Bacteriological Reviews* **28**, 231–295.
- SCHMIDT, J. M. & STANIER, R. Y. (1965). Isolation and characterization of bacteriophages active against stalked bacteria. *Journal of General Microbiology* **39**, 95–107.
- SHAPIRO, L., AGABIAN-KESHISHIAN, N. & BENDIS, I. (1971). Bacterial differentiation. *Science, New York* **173**, 884–892.