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Genetic Recombination in *Rhodopseudomonas capsulata*

(nonsulfur purple photosynthetic bacteria/cell-free filtrate)

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ABSTRACT A system for genetic exchange in *Rhodopseudomonas capsulata* has been discovered. Each genetic marker thus far examined can be transferred, and many strains of *Rps. capsulata* can participate in genetic exchange. The mechanism of gene transfer seems unlike that of any previously described bacterial system, since genes can be transferred by cell-free filtrates, but the vector is resistant to deoxyribonuclease and has a sedimentation constant of about 70 S.

Nonsulfur purple photosynthetic bacteria manifest a remarkable range of metabolic plasticity, especially in regard to alternative modes of energy conversion. Certain species, particularly of the genus *Rhodopseudomonas*, grow rapidly, and in recent years such organisms have been increasingly used in studies on energy conservation processes, membrane formation, and metabolic regulation (1–3). Although biochemical mutants have been used in some of these researches, genetic approaches to elucidation of mechanisms have not been possible hitherto owing to our ignorance of genetic exchange processes in the nonsulfur purple bacteria. This report describes the discovery of a genetic recombination system in *Rhodopseudomonas capsulata*. The genetic transfer appears to be mediated by a novel vector.

MATERIALS AND METHODS

Rhodopseudomonas capsulata, strain "St Louis," and the strains derived from it (Z-1, M1, and M5) have all been described (4); *Rps. capsulata*, strain KB1 was obtained from S. (n) Kaplan. Strains B6, B10, and H9 were isolated from pond and soil samples by standard enrichment techniques. The latter three isolates have been tentatively identified as *Rps. capsulata* on the basis of pigmentation, cell morphology, and especially the characteristic "zig-zag" arrangement of cells in chains.

The medium used throughout was composed of 0.3% Bactopeptone and 0.3% Bacto yeast extract in deionized water; for preparation of solid media, either 0.6 or 1.2% Bacto-agar was used.

Photosynthetic growth was achieved in screw-cap tubes filled to capacity; these were incubated at 35° and illuminated by a bank of three 60-watt lumiline bulbs (General Electric) at a distance of about three inches (7.62 cm).

Cell-free filtrates, capable of transmitting genetic information, were prepared by filtering photosynthetic cultures in early stationary phase (3 to 4 $\times$ 10^9 cells per ml) through 0.45-μm filters made of either cellulose acetate (Gelman) or cellulose nitrate (Millipore).

Abbreviation: CFU, colony-forming units.

A typical assay of gene-transfer activity is performed as follows: 0.2 ml of filtrate from the donor culture is incubated aerobically with 0.2 ml of a recipient culture in early stationary phase for about 20 min at room temperature. The mixture is then diluted appropriately; an aliquot is mixed with 2.5 ml of molten soft agar; and the soft agar is then poured onto a layer of solid medium. Plates are incubated at 30°. If the genetic transfer involves antibiotic resistance, a 6 to 12-hr period of phenotypic expression is allowed before the plates are overlayered with antibiotic-containing soft agar (final antibiotic concentration, about 64 μg/ml).

RESULTS

Several independently isolated strains of *Rhodopseudomonas* species were screened for genetic recombination as follows. First, a rifampicin-resistant mutant and a streptomycin-resistant mutant were selected from each strain. Media were inoculated with pairs of strains to be tested, one member of each pair bearing the streptomycin-resistance marker, the other, refampicin-resistance. After several generations of mixed growth, the frequency of simultaneous resistance to streptomycin and rifampicin was determined and compared to the same frequency measured in singly inoculated control cultures. This procedure revealed a pair of strains, H9 and B10, that consistently gave significantly more rifampicin- and streptomycin-resistant colony-forming units (CFU) than would be expected from the frequency of doubly resistant colony-forming units in the control cultures (Table 1). Tests

TABLE 1. *Gene transfer activity*

Assay mixture*		New phenotype titer rif-str-r CFU per ml $\times$ 10^{-2} †
H9 rif-r	B10 str-r	
Whole culture	None	4
None	Whole culture	18
Whole culture	Whole culture	847
Cell-free filtrate	None	0
Cell-free filtrate	Whole culture	870

* Assay was performed as described in *Methods*, except mixtures were incubated for 1 hr at 25° before dilution and plating. H9 rif-r culture, 3.7 $\times$ 10^9 cells per ml; B10 str-r culture, 2.4 $\times$ 10^9 cells per ml.

† Colonies were counted on plates containing 65 μg/ml of both rifampicin and streptomycin; expressed per ml of either starting culture. Values have been multiplied by 10^{-2}.

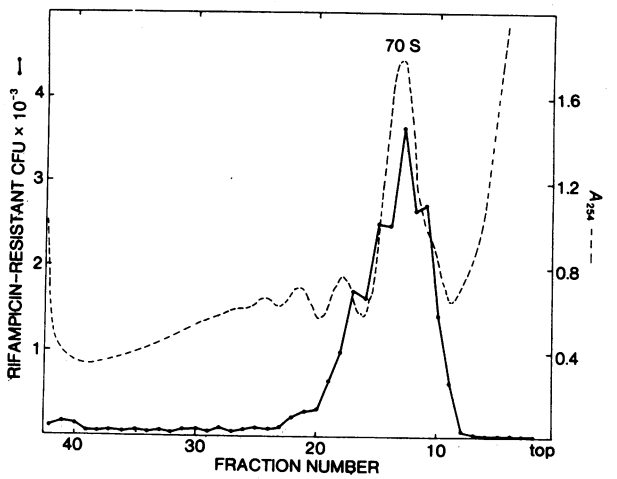

FIG. 1. Sedimentation rate of gene transfer agent. A mixture of cell-free filtrate of an H9 rif-r culture and polyribosomes from *Escherichia coli* was sedimented through a 5–20% sucrose density gradient containing 0.3 mM $MgSO_4$, 0.25 mM $CaCl_2$, 0.0005% gelatin, and 3 mM Tris·HCl (pH 7.3) by centrifugation in a Beckman SW 50L rotor at 39,000 rpm for 45 min at 4°. Gradients were fractionated with an ISCO Density Gradient Fractionator, model 183, and the A_{254} was recorded on an ISCO UV analyzer, model 224. Fractions were assayed for gene transfer activity essentially as described in *Methods*.

designed to examine the mechanism of the presumptive genetic recombination revealed that the transfer is mediated by a filterable agent (Table 1) that is DNase resistant (Table 2). Although these results suggest that transduction might be the mechanism of the genetic exchange, the 70S sedimentation coefficient of the gene transfer agent (Fig. 1) is much lower than that for any known transducing phage (controls demonstrated that the gene-transferring activity sediments at about the same rate in the absence of marker ribosomes). The gene transfer activity is not inducible by mitomycin C, no plaque formation or liquid culture lysis has been observed, and virus-like particles could not be detected in preliminary electron microscopic examinations. However, these tests do not exclude the existence of a small transducing phage.

The genetic exchange system described has been effective in transferring each marker examined thus far, including: (*a*) rifampicin resistance, (*b*) streptomycin resistance, (*c*) the wild-type allele of a lesion that is responsible for the inactive NADH dehydrogenase of strain M1 [ref. 5; M1 was formerly designated Z-1 (aer-13); see Table 3], (*d*) the wild-type alleles for each of two lesions that result in the loss of terminal oxidase activities in strain M5 (see Table 4), and (*e*) the wild-type allele for a mutation that results in an inability to grow with thymine as sole nitrogen source. It thus seems likely that any region of the chromosome may be transferred.

TABLE 2. *DNase resistance of gene transfer activity*

H9 rif-r filtrate	λ[³H]DNA	DNase	Trichloroacetic acid ppt cpm*	rif-r CFU per ml × 10^{-2}
+	+	−	1263	183
+	+	+	40	177
−	+	−	1083	4

* Each complete reaction mixture consisted of: 0.2 ml of cell-free filtrate of H9 rif-r culture; 0.2 ml of 6 mM $MgCl_2$, 5 mM $CaCl_2$, 0.01% gelatin, 60 mM Tris·HCl, pH 7.3; 0.05 ml of ³H-labeled bacteriophage λ DNA (38 μg/ml); 0.05 ml of pancreatic DNase (10 mg/ml) (Sigma). The mixtures were incubated for 7.3 hr at 30°, at which time 0.1 ml was removed from each mixture for a gene-transfer activity titer, and 0.4 ml from each mixture was precipitated with 0.5 ml of 10% trichloroacetic acid and 0.1 ml of bovine-serum albumin (3 mg/ml) for determination of acid-precipitable radioactivity.

TABLE 3. *Donor origin of the induced recombinants*

Donor filtrate	Recipient cells	Plating conditions	CFU/ml × 10^{-1}*
H9 rif-r	B10 str-r	Rifampicin and streptomycin	~3000
H9 (rif-s)	B10 str-r	Rifampicin and streptomycin	26
None	B10 str-r	Rifampicin and streptomycin	36
H9 rif-r	M1	Aerobic, dark	364
H9 (rif-s)	M1	Aerobic, dark	368
None	M1	Aerobic, dark	2
H9 rif-r	None	Aerobic, dark	0
H9 (rif-s)	None	Aerobic, dark	0

* Gene transfer was performed as described in *Methods;* see *text* for description of strains.

The experiment shown in Table 3 was conducted to rule out the possibility that what appeared to be a transfer of genetic information might actually be nonspecific mutagenesis. It is apparent that rifampicin-resistant recombinants are recovered only if the rifampicin-resistance marker is carried by the donor strain. The controls show that both the rifampicin-sensitive parent, H9(rif-s), and the resistant strain derived from it, H9 rif-r, are active in the transfer of genes capable of conferring the wild-type phenotype on strain M1, a mutant that is unable to grow aerobically in the dark.

The observation that the gene transfer system discovered in strains H9 and B10 is compatible with strain St. Louis enhances the utility of the system. For example, Marrs and Gest (4) have reasoned that strain M5, a cytochrome oxidase negative derivative of strain St. Louis, probably contains two defects in its respiratory electron transport system, both of which are necessary to produce the observed inability of M5 to grow aerobically in darkness. Table 4 gives the results of an experiment that proves that strain M5 does indeed contain two genetically distinct lesions, as anticipated. If the obligate photoheterotrophic phenotype of strain M5 was caused by

TABLE 4. *Selection of aerobic recombinants from strain M5*

Filtrate	Cells	CFU per ml × 10^{-2}* Nadi positive	Nadi negative
H9 rif-r	M5	115	63
None	M5	1	0
H9 rif-r	None	0	0

* Gene transfer performed as described in *Methods;* colony-forming units were determined under aerobic, dark plating conditions, and these colonies were tested for cytochrome oxidase activity by the Nadi reaction.

TABLE 5. *Ability of various strains of* Rps. capsulata *to participate in genetic recombination*

Exp.	Filtrate	Cells	rif-r CFU per ml × 10^{-2}*
1	None	B10 str-r	5
	Z-1 rif-r	B10 str-r	40
	B10 rif-r	B10 str-r	231
	H9 rif-r	B10 str-r	116
	None	H9 (rif-s)	6
	Z-1 rif-r	H9 (rif-s)	7
	B10 rif-r	H9 (rif-s)	5
	H9 rif-r	H9 (rif-s)	7
	None	Z-1 (rif-s)	11
	Z-1 rif-r	Z-1 (rif-s)	31
	B10 rif-r	Z-1 (rif-s)	139
	H9 rif-r	Z-1 (rif-s)	50
2	None	B10 str-r	33
	KB1 rif-r	B10 str-r	32
	B6 rif-r	B10 str-r	24
	None	KB1 (rif-s)	26
	H9 rif-r	KB1 (rif-s)	91
	None	B6 (rif-s)	27
	H9 rif-r	B6 (rif-s)	320

* Gene transfer assays performed as described in *Methods;* see *text.*

only one genetic lesion, then recombination should produce only one (major) class of cells able to grow under aerobic conditions. Two types of recombinants that have regained the capacity for aerobic growth are, in fact, found: a class that has regained cytochrome oxidase activity (as measured by the Nadi reaction; see ref. 4) and one that remains cytochrome oxidase negative.

Most strains of *Rps. capsulata* thus far examined can participate to some extent in genetic exchange with other strains (Table 5). Strain B10 can send and receive genetic information, and is self-fertile; strain H9 can transmit but cannot receive; B6 cannot transmit but can receive genes; and KB1 cannot send, but does appear to receive genetic information at a low efficiency.

DISCUSSION

The observations described demonstrate the existence of a mechanism for genetic exchange in *Rps. capsulata.* The exchange mechanism appears to be unusual, since the sedimentation value of the gene transfer agent is unlike any previously described DNase-resistant vector of bacterial genes. A possible interpretation that is consistent with our observations is that the gene transfer agent may be a type of sex pilus that is released into the medium in a genetically active form (see ref. 6). Whatever the physical nature of the gene transfer agent, the system can now be used for initiating studies of the genetic map of *Rps. capsulata,* for constructing useful strains for investigations of biochemical mechanisms, and for the resolution of mutant strains suspected of carrying more than one mutation.

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