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Conjugational Transfer Systems of *Rhodopseudomonas capsulata* Mediated by R Plasmids

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Abstract. Plasmids RP1, R68.45 and RP4::Mu cts 61 were transferred into *Rhodopseudomonas capsulata* from *Escherichia coli*. The frequency of intraspecies transfer of these plasmids in *R. capsulata* was $10^{-4}-10^{-5}$ per donor. The plasmids also mobilized chromosomal genes at a low frequency. Phototrophic recombinants from matings between recipient strains defective in the photosynthetic-apparatus and wild type donors were obtained at a frequency of $10^{-7}-10^{-8}$ per donor.

Key words: Conjugational transfer – R plasmids – *Rhodopseudomonas capsulata*

Photosynthetic bacteria have been used to study the organization and differentiation of membranes in response to various external factors such as light intensity, oxygen tension and nutrition (reviewed in Oelze and Drews 1972; Kaplan 1978; Drews 1978). Major efforts at the present are to study the process at the molecular level and to examine the genetic control of the formation of the photosynthetic-apparatus (Saunders 1978). An effective genetic exchange system is of paramount importance in order to map the genes responsible for the expression of the photosynthetic-apparatus and to study the genetic control. In *Rhodopseudomonas capsulata*, it has been shown by cotransfer analyses with a gene transfer agent and ratio test crosses that loci for five carotenoid and two bacteriochlorophyll genes are closely linked within a region which is estimated to be less than 1% of the genome (Yen and Marrs 1976). The same genetical transfer system has also restored a white mutant for the synthesis of bacteriochlorophyll and of reaction center and light harvesting B 870-proteins (Drews et al. 1976).

Because of the small size (about 3.6×10^6 daltons) of the DNA transferred by gene transfer agent (Solioz and Marrs 1977), we are searching for other genetical transfer systems. Conjugation and chromosomal transfer in *Rhodopseudomonas sphaeroides* mediated by W and P group plasmids have recently been reported (Sistrom 1977; Miller and Kaplan 1978; Marrs 1979, Tucker and Pemberton 1979a, b). We report here the introduction of the P group plasmids RP1 (Grinsted et al. 1972) and R 68.45 (Haas and Holloway 1976) and a hybrid plasmid RP 4::Mu cts 61 (Dénarié et al. 1977; Van Vliet et al. 1978) into *R. capsulata*. All three plasmids carry resistance genes for kanamycin, ampicillin and tetracycline. These plasmids were tested for their ability to mobilize the chromosome of *R. capsulata* to transfer the genes for the bacteriochlorophyll-protein complexes to mutants which are defective in the formation of the photosynthetic-apparatus.

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Materials and Methods

Bacterial Strains and Plasmids. The wild type *Rhodopseudomonas capsulata*, strain 37b4 [Deutsche Sammlung von Mikroorganismen (DSM), Göttingen, number 938] and mutants derived from this strain were cultivated anaerobically in light on malate medium with (0.05%) or without yeast extract (Drews 1965). The media for growing auxotrophic mutants were supplemented with the respective amino acids. The following strains of *Escherichia coli* were cultivated on nutrient broth: 528, rK^-, mK^-; 327, met, thy, leu, nal (RP1); C 600, thr-1, leu-6, thi-1 (RP4::Mu cts 61); 528-1, 528 (R68.45). Strain 528-1 was constructed by tansformation of strain 528 with R 68.45 plasmid DNA from WS 27. The WS 27 *Rhodopseudomonas sphaeroides* strain (his, pro, R 68.45) was obtained from Dr. W. Sistrom. The *E. coli* strains 528 and 327 were provided by Dr. H. Saedler. The C 600 strain with the RP 4::Mu cts 61 was a gift from Dr. J. Schell. The other *R. capsulata* strains have been described before or have been obtained by mutagenic treatment with N-methyl-nitrosoguanidine in this laboratory (Drews et al. 1976; Feick and Drews 1978).

Analytical Procedures. Absorption spectra of membranes were measured in a Cary 14 R spectrophotometer (Varian). The preparation of crude membranes has been described elsewhere (Feick and Drews 1978). Membrane proteins were separated by SDS polyacrylamide gel electrophoresis on 1 mm slab gels (Laemmli 1970) using a 11.5–16.5% continuous gradient of acrylamide.

Preparation of Plasmid DNA. A procedure modified from Sharp et al. (1972) was used for the preparation of large quantities of purified plasmid DNA. From 1 l of stationary phase culture, the cells were harvested and resuspended in 25 ml TES buffer (0.05 M Tris-HCl, 0.005 M EDTA, 0.05 M NaCl, pH 8.5). Then 25 ml of sucrose-lysozyme solution (20% sucrose in TES buffer and 50 mg lysozyme) and 0.25 ml ribonuclease (Boehringer) (20 mg/ml in 50 mM sodium acetate, pH 5.2, heat treated at 80°C for 10 min) were added, and the mixture was incubated in a waterbath at 37°C for 20 min with gentle stirring. Afterwards 25 ml of laurylsarcosine (2% solution in TES buffer) was added and mixed vigorously for 4–5 min. The total mixture was then cooled in an ice bath and sheared with plastic syringe. The chromosomal DNA was then denatured by raising the pH to 12.3 with 4 N NaOH and kept at this pH for 3 min before lowering the pH to 8.5 by adding 2 N HCl. The concentration of Na^+-ions was adjusted to 0.3 M by the addition of NaCl solution (5 M). This mixture was then added to 10 g nitrocellulose (washed twice with TES buffer) and mixed for 1 h at 4°C. The mixture was then centrifuged (7000 rpm for 15 min), and the supernatant was filtered through a glass-wool column and layered onto 3 ml saturated CsCl solution and centrifuged in a Beckman Ti 60 rotor for 15 h at 15,000 rpm, 4°C. 30 ml from the bottom of each tube was collected, the density was adjusted with CsCl to 1.55 (refractive index of 1.3866–

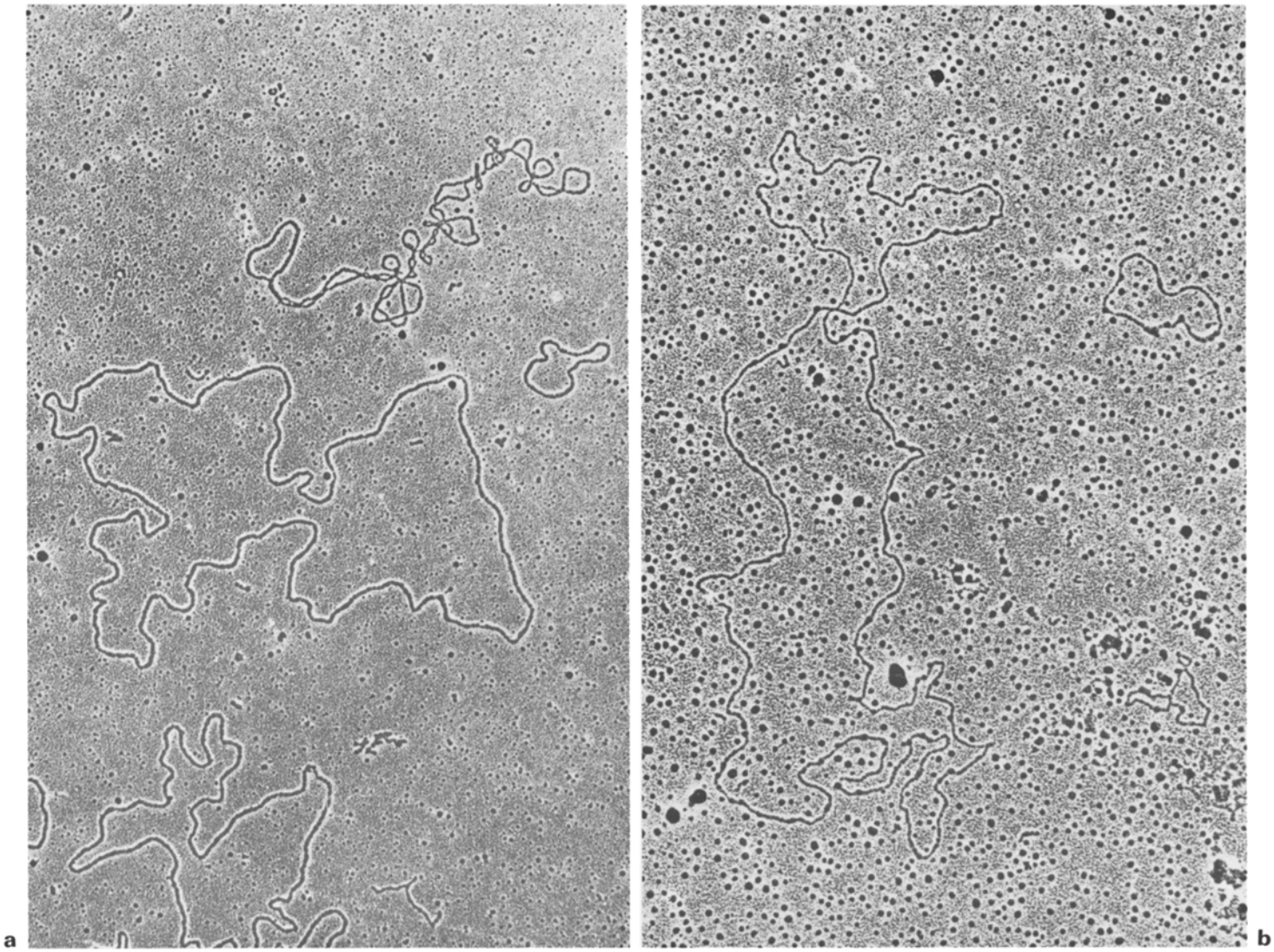

Fig. 1. Electron micrographs by palladium-platinum shadowing method of (**a**) RP 1 plasmid DNA (both open and twisted form) (**b**) R 68.45 plasmid DNA. Replicating form of fd DNA was used as size standard. The DNA length was measured with a Hewlett-Packard digital computer by a program from H. Broda. The plasmid DNA were isolated from *Rhodopseudomonas capsulata*

1.391) and 500 µl/ml ethidium bromide was added. The density equilibrium centrifugation was performed with a Beckman Ti 50 rotor (40,000 rpm for 40 h, 12° C). The lower plasmid containing band was collected, extracted twice with CsCl-saturated n-butanol and once with CsCl-saturated n-butanol:isopropylalcohol (1:1) and was dialysed against 10 mM Tris-HCl, 1 mM EDTA, pH 8.2.

Antibiotics. Rifampicin was dissolved in absolute methanol and used without further sterilization. All other antibiotics were dissolved in distilled water and filter-sterilized (Millipore, 0.30 µm) before use. Final concentrations of antibiotics used for all strains were: kanamycin sulfate, Km, 25 µg/ml (Sigma); ampicillin trihydrate, Ap, 100 µg/ml (Hoechst); tetracycline hydrochloride, Tc, 10 µg/ml (Sigma); streptomycin sulfate, Sm, 250 µg/ml (Sigma) and rifampicin, Rif, 50 µg/ml (Boehringer).

Endonuclease Digestion and Agarose Gel Electrophoresis. Two units of Hind III endonuclease (Boehringer) were used to digest 1 µg DNA in the following buffer: 60 mM NaCl, 7 mM $MgCl_2$, 10 mM Tris-HCl, 100 µg/ml bovine serum albumine, pH 7.4 at 37° C for 1 h.

Agarose gel electrophoresis was performed on 1.2% agarose gel (40 mM Tris-HCl, 5 mM sodium acetate, 1 mM EDTA, pH 7.8 buffer) with 10 µg/ml ethidium bromide present. The gels were photographed in UV light (254 nm) with Polaroid MP 3-Camera equipped with an orange filter.

Electron Microscopy of DNA. DNA was spread by the Kleinschmidt drop diffusion method (Lang and Mitani 1970) on Parlodion-coated grids (200 mesh, Veco) and shadowed with palladium-platinum wire (Balzer-Bedampfungsanlage BA 3). Replicating from fd DNA was used as size standard. A Philips EM 201 C microscope was used.

Mating Procedure. All matings were done on agar plates, using two methods. (i) The agar overlay method: a soft agar overlay containing antibiotics was added after allowing time for mating. (ii) The spread mating method in which all the cells were washed off from the mating agar surface before diluting and plating on selective agar plates. The latter method was preferred if further testing of the transconjugants by replica plating was needed. For both types of mating the density of donor and recipient was 2×10^9 cells per ml and a donor:recipient ratio of 1:1 was used. We normally allowed mating overnight (about 15 h) before plating the cells on plates with antibiotics. Long mating periods (more than 9 h) might perturb the transfer frequencies due to differential growth of the transconjugants. However, for the purpose of the experiments here, we were mainly interested in the relative frequencies of transfer of chromosomal markers and we did not attempt to evaluate absolute transfer frequencies.

Results

Transfer of R Plasmids from Escherichia coli to Rhodopseudomonas capsulata. Overnight cultures of *E. coli* donor strains and *R. capsulata* wild type strains were spread mated on

Table 1. Intraspecies Transfer of Plasmids in *Rhodopseudomonas capsulata*

Donor/Plasmid	Recipient	Transfer frequency of transconjugants per donor[a]		
		Plasmid transfer[b] ($\times 10^5$)[b]	Chromosomal recombinants ($\times 10^8$)[c]	Chromosomal recombinants/ plasmid transfer (%)
37b4 Leu$^-$ Ileu$^-$ Rif^r RP1	Y 5 Sm^r Pho$^-$	1.8	5.0	0.28
37b4 Leu$^-$ Ileu$^-$ Rif^r RP4::Mu cts 61		58	81	0.14
37b4 Thr$^-$ Leu$^-$ Rif^r R 68.45		1.4	55	3.9
37b4 Leu$^-$ Ileu$^-$ Rif^r RP1	A1a$^+$ Sm^r Crt$^-$	13	92	0.71
37b4 Leu$^-$ Ileu$^-$ Rif^r RP4::Mu cts 61		65	78	0.12
37b4 Thr$^-$ Leu$^-$ Rif^r R 68.45		1.2	50	4.2

[a] Mating was as described in methods. Kanamycin resistant transconjugants were tested by replica plating on medium containing rifampicin to rule out that they were revertants from donors. Furthermore, the reversion rate of Y 5 to wild type was found to be less than 10^{-9} in normal medium without kanamycin. With the addition of kanamycin (25 µg/ml), we did not observe any spontaneous resistant colonies from the two recipient strains

[b] Plasmid transfer frequency is given as the number of kanamycin resistant transconjugant colonies per donor

[c] Chromosomal recombinants having the Km^r Pho$^+$ and Km^r Crt$^+$ characteristics were scored for Y 5 and A1a$^+$ respectively

nutrient broth agar medium followed by phototrophic growth on minimal malate agar medium supplemented with kanamycin. The frequencies of transfer of the selected marker were 6×10^{-3}, 7×10^{-4} and 7×10^{-4} per donor for the plasmids RP4::Mu cts 61, RP1 and R 68.45 respectively and transconjugants were also resistant to tetracycline and ampicillin. Control plates of *R. capsulata* wild type showed no spontaneous kanamycin resistant mutant colonies. The three plasmids could be isolated from *R. capsulata* transconjugants and detected as satellite bands in ethidium bromide-caesium chloride density gradient centrifugation. The plasmids were transformed into *E. coli* strain 528 and the transformants showed the expected antibiotic resistances. Further characterization of the plasmids from RP 1 and R 68.45 transconjugants by electron microscopy gave plasmid sizes of 40 and 38 megadaltons respectively (Fig. 1). These results confirmed that the antibiotic resistances were plasmid-borne rather than being spontaneous resistance mutations in the recipients. We were not able to detect any wild type plasmid from our *R. capsulata* 37b4 wild type strain using our plasmid isolation procedure.

Chromosome Mobilisation in R. capsulata. Preliminary experiments showed that late log phase cells gave the best frequencies of plasmid transfer. Phototrophic strains carrying the plasmids RP1, RP4; Mu cts 61 or R 68.45 were used as donors. Two strains carrying mutations in the photosynthesis system were used as recipients: (1) strain Y 5, a phototrophic negative mutant, which has the light harvesting bacteriochlorophyll-protein complex B 800–850 and carotenoids, but lacks an effective reaction center and light harvesting complex B 870, (2) A 1a$^+$, which is a phototrophic positive blue green mutant but lacks both carotenoids and light harvesting complex B 800–850 (Feick and Drews 1978). The former fails to grow in the light under anaerobic conditions and the latter can be recognized by its absorption spectrum.

The donor and recipient strains were mated (Table 1). The donors were counter-selected with two amino acid auxotrophies and streptomycin (Table 1). The resulting kanamycin resistant transconjugants must have been derived from the recipient strain because they were all rifampicin sensitive whereas the donor strains were rifampicin resistant. As Table 1 shows, between 0.1% and 4% of the kanamycin resistant transconjugants had a wild type phenotype for the photosynthesis system. Ten of the Y 5-derived phototrophic transconjugants were examined further. They showed a wild type absorption spectrum (data not shown) and had restored a wild type membrane polypeptide pattern (Fig. 2). The Y 5 recipient strain reverts at a very low frequency (less than 10^{-9}) to phototrophy and the phototrophic negative kanamycin resistant transconjugants are also stable; thus, it seems that the phototrophic positive transconjugants are indeed due to chromosome mobilisation by the R factors. R 68.45 produces about a tenfold higher number of photosynthetic wild type transconjugants than RP 1 or RP4::Mu cts 61 (Table 1).

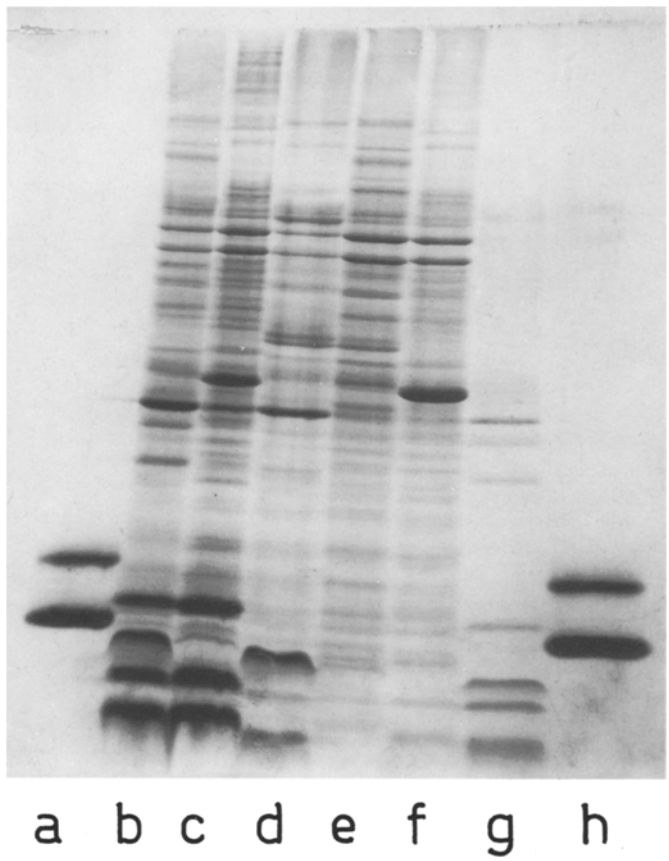

Fig. 2. Polyacrylamide gel electrophoresis of membranes from *Rhodopseudomonas capsulata* by Slab gels (see Methods). *a* and *h* β-lacto myoglobin (19,400) and cytochrome *c* (12,500) as markers; *b* strain 37b4 (wild type); *c* strain Y5; *d* A1a$^+$pho$^+$; *e* 37b4 pho$^-$; *f* A1a$^+$pho$^-$. *g* Phototroph positive transconjugant of Y5

Discussion

Hu and Marrs (1979) reported that *Rhodopseudomonas capsulata* strain BH 9 contains at least two plasmids of sizes 74 and 94 megadaltons. We did not detect any plasmids in our wild type strain 37b4, but this might due to the plasmid isolation procedure that we used.

The transfer frequency of RP 1 between our *R. capsulata* strains was low ($10^{-4}-10^{-5}$ per donor, Table 1) compared to that reported in *R. sphaeroides* ($10^{-2}-10^{-3}$ per donor, Miller and Kaplan 1978). However, the frequency of chromosomal transconjugants per plasmid transconjugants was relatively high (0.1 – 4%, Table 1). One possible explanation for these observations is that plasmid establishment in a recipient cell after transfer is inefficient, so that recovery of a kanamycin resistant transconjugant is a gross underestimate of the true plasmid transfer frequency. The plasmid R 68.45 was about tenfold better than the other two plasmids in chromosome mobilization; it gives an increased mobilisation in several other bacterial species also (Holloway 1979). The frequency of chromosomal transconjugants (5×10^{-7} per donor for R 68.45, Table 1) is still rather low for genetic analysis. However, improved mating conditions might lead to a somewhat higher frequency.

It is interesting that RP 4::Mu cts 61 shows no zygotic induction in *R. capsulata*, transferring, if anything, somewhat better than RP 1 (Table 1). This is similar to the situation in *Pseudomonas solanacearum* (Boucher et al 1977). The trivial possibility that Mu has suffered a deletion seems to be ruled out by the fact that growth of *R. capsulata* (RP 4::Mu cts 61) at 37° C led to production of Mu phages that would plate on *E. coli* (P. L. Yu, unpublished results). It is clear from Table 1 that RP 4::Mu cts 61 is no better than RP 1 in mobilising the Rhodopseudomonas chromosome.

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References

Boucher C, Bergeron B, de Bertalmio MB, Dénarié J (1977) Introduction of bacteriophage Mu into *Pseudomonas solanacearum* and *Rhizobium meliloti* using the R factor RP 4. J Gen Microbiol 98:253 – 263

Dénarié J, Rosenberg C, Bergeron B, Boucher C, Michel M, Barate de Bertalmio M (1977) Potential of RP 4: Mu plasmids for in vivo genetic engineering of gram-negative bacteria. In: Bukhari AI, Shapiro JA, Adhya SL (eds) DNA insertion elements, plasmids and episomes. Cold Spring Harbor Symposium, New York, pp 507 – 520

Drews G (1965) Die Isolierung schwefelfreier Purpurbakterien. Zbl Bakt I Supplementheft 1:170 – 178

Drews G, Dierstein R, Schumacher A (1976) Genetic transfer of the capacity to form bacteriochlorophyll-protein complexes in *Rhodopseudomonas capsulata*. FEBS Lett 68:132 – 136

Drews G (1978) Structure and development of the membrane system of photosynthetic bacteria. Curr Top Bioenerg 8B:161 – 207

Feick R, Drews G (1978) Isolation and characterization of light harvesting bacteriochlorophyll-protein complexes from *Rhodopseudomonas capsulata*. Biochim Biophys Acta 501:499 – 513

Grinsted J, Saunders JR, Ingram LC, Sykes RB, Richmond MH (1972) Properties of an R factor which originated in *Pseudomonas aeruginosa* 1822. J Bacteriol 110:529 – 537

Haas D, Holloway BW (1976) R factor variants with enhanced sex factor activity in *Pseudomonas aeruginosa*. Mol Gen Genet 144:243 – 251

Holloway BW (1979) Plasmids that mobilize bacterial chromosome. Plasmid 2:1 – 19

Hu NT, Marrs BL (1979) Characterization of the Plasmid DNAs of *Rhodopseudomonas capsulata*. Arch Microbiol 121:61 – 69

Kaplan S (1978) Control and kinetics of photosynthetic membrane development. In: Clayton RK, Sistrom WR (eds) The photosynthetic bacteria. Plenum Press, New York, pp 809 – 839

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T 4. Nature (Lond) 227:680 – 685

Lang D, Mitani M (1970) Simplified quantitative electron microscopy of biopolymers. Biopolymers 9:373 – 379

Marrs B (1979) Genetics of photosynthetic bacteria. III. Int Symp Photosynth Prokaryotes, Nichols JM ed, C 1 Oxford

Miller L, Kaplan S (1978) Plasmid transfer and expression in *Rhodopseudomonas sphaeroides*. Arch Biochem Biophys 187:229 – 234

Oelze J, Drews G (1972) Membranes of photosynthetic bacteria. Biochim Biophys Acta 265:209 – 239

Saunders VA (1978) Genetics of *Rhodospirillaceae*. Microbiol Rev 42:357 – 384

Sharp PA, Hsu MT, Ohtsubo E, Davidson N (1972) Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. I. Structure of F-prime factors. J Mol Biol 71:471 – 479

Sistrom WR (1977) Transfer of chromosomal genes mediated by plasmid R 68.45 in *Rhodopseudomonas sphaeroides*. J Bacteriol 131:526 – 532

Solioz M, Marrs B (1977) The gene transfer agent of *Rhodopseudomonas capsulata*: Purification and characterization of its nucleic acid. Arch Biochem Biophys 181:300 – 307

Tucker WT, Pemberton JM (1979a) Conjugation and chromosome transfer in *Rhodopseudomonas sphaeroides* mediated by W and P group plasmids. FEMS Lett Microbiol 5:173 – 176

Tucker WT, Pemberton JM (1979b) The introduction of RP 4::$Mu_{cts\,62}$ into *Rhodopseudomonas sphaeroides*. FEMS Microbiol Lett 5:215 – 217

Van Vliet F, Silva B, Van Montagu M, Schell J (1978) Transfer of RP 4::Mu plasmids to *Agrobacterium tumefaciens*. Plasmid 1:446 – 455

Yen HC, Marrs B (1976) Map of genes for carotenoid and bacteriochlorophyll biosynthesis in *Rhodopseudomonas capsulata*. J Bacteriol 126:619 – 629

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