JOURNAL OF BACTERIOLOGY, June 1984, p. 1094–1103
0021-9193/84/061094-10$02.00/0

Vol. 158, No. 3

Plasmid Rearrangements in the Photosynthetic Bacterium *Rhodopseudomonas sphaeroides*

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Received 26 October 1983/Accepted 19 March 1984

Mu d1(Ap *lac*) was introduced into the photosynthetic bacterium *Rhodopseudomonas sphaeroides* 2.4.1. via the R-plasmid R751 in an attempt to isolate fusion derivatives involving photosynthetic operons. The selection system is potentially very powerful since *R. sphaeroides* is normally Lac negative. Among the exconjugants, photosynthesis-deficient mutants were recovered, some of which had elevated β-galactosidase levels. Among the mutants examined, β-galactosidase expression was linked exclusively to R751. Many of the photosynthesis-deficient mutants were found to have alterations in their indigenous plasmids which apparently involved the exchange of DNA from one plasmid to another. Southern blot analysis revealed that there are extensive DNA sequences which are shared by the two plasmids that are involved in the rearrangements and that no exogenous DNA sequences appear to be involved. It was further discovered that plasmid rearrangement is a general phenomenon which can occur spontaneously in *R. sphaeroides* 2.4.1 and shows a high correlation with a photosynthesis minus phenotype.

The photosynthetic bacterium *Rhodopseudomonas sphaeroides* possesses numerous physiological capabilities and has therefore been the object of considerable experimental interest. One interest of our laboratory is the development of the intracytoplasmic membrane (ICM), the structure that houses the pigments and proteins responsible for the capture of light energy and its conversion (27, 28, 32) into chemical energy. The ICM is not present in chemoheterotrophically (aerobically) grown cells, but its synthesis can be induced by lowering the oxygen tension below 5% (17). Once induced, the amount and composition of the ICM are regulated by the incident light intensity (1, 35). Under high-light conditions, both the total amount of ICM and the ratio of light-harvesting to photochemical reaction center components is low relative to the ratio obtained from cells grown under low-light conditions (1). The dynamics of membrane induction and synthesis as well as the gratuitous nature of the ICM in *R. sphaeroides* provide an interesting experimental system in which to study the control of gene regulation of a differentiating entity in an easily controlled procaryotic system. Our laboratory has been investigating aspects of the physical assembly of the ICM (for a review, see reference 17) and we have begun to establish genetic tools to probe the nature of the genetic regulation of the ICM and its components (9, 24).

Several investigators have examined plasmids of the purple nonsulfur bacteria in an effort to determine whether any of the activities associated with photosynthesis are plasmid determined (12, 13, 33, 37). In particular, Saunders and co-workers (33) found three plasmids in *R. sphaeroides* 2.4.1 of sizes 42, 99, and 114 kilobases (kb). The 42- and 99-kb plasmids have a buoyant density of 1.717 g/cm^3 (58% G+C), and the 114-kb plasmid has a buoyant density of 1.724 g/cm^3 (65% G+C). After attempting to cure cells of these plasmids with sodium dodecyl sulfate, these investigators isolated a photosynthesis-deficient (Pho$^-$) mutant that was missing the 42-kb plasmid but which had a new plasmid species of 50 kb (buoyant density, 1.717 g/cm^3). These observations prompted Saunders and co-workers to suggest that a photosynthetic function encoded on the 42-kb plasmid had been inactivated by insertional mutagenesis, although the precise mechanism and molecular origin of the insertion were not discussed.

Because many investigators have used Mu d1(Ap *lac*) (3) as a tool in understanding transcriptional regulation of numerous bacterial operons, we felt that its application to the study of ICM regulation was a promising approach in light of our previous finding that the *lac* operon could be expressed in *R. sphaeroides* (24). We observed that the introduction of Mu d1(Ap *lac*) into *R. sphaeroides* via R-plasmid R751 resulted in the appearance of photosynthetically deficient (Pho$^-$) mutants. Although our initial premise was that Mu d1(Ap *lac*) was acting in the expected manner, we subsequently determined that many of the *R. sphaeroides* Pho$^-$ mutants resulting from the introduction of Mu d1(Ap *lac*) had an altered indigenous plasmid complement. We have further determined that the induction of at least some of the Pho$^-$ mutants did not physically involve Mu d1(Ap *lac*) itself. Instead, we hypothesize that a *trans*-acting activity of bacteriophage Mu destabilizes an endogenous genetic element located on one or more of the endogenous *R. sphaeroides* plasmids.

(Preliminary results of this work were presented at the 82nd Annual Meeting of the American Society for Microbiology, Atlanta, Georgia, 7 to 12 March, 1982.)

MATERIALS AND METHODS

Bacterial strains, plasmids, bacteriophages, and growth conditions. Table 1 lists the strains, plasmids, and bacteriophages used in this study.

R. sphaeroides strains were grown in Sistrom A medium (35) at 30°C. Cells were grown chemoheterotrophically in 125-ml growth flasks in a shaking (250 rpm) New Brunswick water bath. Photoheterotrophic growth was accomplished by incubation of cells in filled screw-capped tubes and illumination with incandescent light (General Electric) at 7.5 W/m^2 as measured through a Corning colored glass filter (no. 7-69). When necessary, controlled oxygen levels were achieved by sparging 800-ml cultures with 600 ml of a mixture of O_2 and air for high O_2 tension and with air, N_2, and CO_2 for low O_2 tensions per min. Oxygen levels were

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TABLE 1. Bacterial strains, plasmids, and bacteriophages used in this study

Designation	Genotype or phenotype	Source or reference
E. coli		
FN22	UC4288(pUI2)	This work
FN23	UC4288(pUI3)	This work
FN52	JC3272(pUI31)	This work
FN56	JC3272(pUI35)	This work
FN59	JC3272(pUI38)	This work
HB101	*pro leu thi lacY str recA hsdR hsdM*	Laboratory strain
JC3272	*his lys trp Δlac-74 rpsL*	G. Cornelius
JC5466	*trp his recA56 spc*	J. Denarie
MA1103	Mu dl(Ap *lac*) *araB*::Mu cts *araD139* (*proAB lacIPOZYA*) XIII	3
MC4100	*araD139 ΔlacU169 rpsL thi*	3
MN1	MC4100::Mu dl(Ap *lac* Km)	This work
UC4200	JC3272(R751)	Laboratory strain
UC4300	HB101(pRK290)	6
UC4400	HB101(pRK2013)	6
UC3014	JC5466(pGMI4)	J. Denarie
UC4288	Δ(*lac-pro*)	Laboratory strain
R. sphaeroides		
2.4.1	Wild type, Glu°, LacZ°[a]	W. R. Sistrom
L1	2.4.1(pUI38), LacZ$^+$ (5.5)[b], Pho (wild type)[c], Tp^r Km^r	This work (FN22 × 2.4.1)
L14	2.4.1(pUI2), LacZ$^+$ (15), Pho (B875$^-$), Tp^r	This work (FN22 × 2.4.1)
T14	2.4.1(pRK290) LacZ$^-$ (0), Pho (B875$^-$), Tc^r	This work (UC4400 × UC4300 × L14)
L19	2.4.1(pUI2), LacZ$^+$ (44), Pho (B800-850/ B875), Tp^r	This work (FN22 × 2.4.1)
T19	2.4.1(pRK290), LacZ$^-$ (0), Pho (wild type)[d], Tc^r	This work (UC4400 × UC4300 × L19)
T19-1	2.4.1, LacZ$^-$ (0), Pho (wild type)	This work; isolated as a spontaneous LacZ on an X-gal plate
L20	2.4.1(pUI2), LacZ$^+$ (37), Pho (B800-850$^-$), Tp^r	This work (FN22 × 2.4.1)
T20	2.4.1(pRK290), LacZ$^-$ (0), Pho (wild type), Tc^r	This work (UC4400 × UC4300 × L20)
L33	2.4.1(pUI38), LacZ$^+$ (5.5), Pho (B875$^-$), Tp^r Km^r	This work (FN59 × 2.4.1)
T33	2.4.1(pRK290), LacZ$^-$ (0), Pho (B875), Tc^r	This work (UC4400 × UC4300 × L33)
CU1022	2.4.1., Pho (B800-850/ B875)	This work; Met$^+$ exconjugant of CU620 × CU622
Plasmids		
pMB041	pBR322::*Hin*dIII-*Pst*I clone of Mu dl(Ap *lac*)	M. B. O'Connor
pRK290	IncP1; Tc^r	6
pRK2013	IncP1; Km^r	6
RP4	IncP1; Tc^r Km^r Ap^r[e]	Laboratory stock
R751	IncP1; Tp^r	R. Hedges
pGMI4	RP4::Mu *cts62*; Tc^r Km^r Ap^r	J. Denarie
pUI2	R751::Mu dl(Ap *lac*);	This work
pUI3	R751::Mu dl(Ap *lac*); Tp^r Ap^r	This work
pUI31	R751::Mu dl(Ap *lac*); Tp^r Ap^r Km^r	This work
pUI35	R751::Mu dl(Ap *lac* Km); Tp^r Ap^r Km^r	This work
pUI38	R751::Mu dl(Ap *lac* Km); Tp^r Ap^r Km^r	This work
pRS2.4.1e	42-kb plasmid of *R. sphaeroides* 2.4.1.	This work
Bacteriophages[f]		
Mu *cts62*	Variant that is temperature sensitive for lytic induction	Laboratory stock
Mu dl(Ap^r *lac*)	*lacZYA* Ap^r	3
Mu *cts62* pf7701Δ 445-3[g]	Km^r Gin$^-$ Mom$^-$	C. Thompson, M. Howe
Mu dl(Ap^r *lac* Km^r)	*lacZYA* Ap^r Km^r	This work

monitored with a Yellow Springs Instruments Co. oxygen meter (model 54).

Escherichia coli strains were grown in LB (21) medium supplemented with glucose. In certain genetic crosses, selection was made on M9 (21) minimal glucose agar medium.

Matings. *E. coli* matings were performed by mixing in a 17-ml test tube 0.3 ml of donor and 0.3 ml of recipient, incubating without agitation at 37°C for 3 h, and then plating 0.1 ml of the mixture on selective agar medium. We performed *E. coli-R. sphaeroides* and *R. sphaeroides-R. sphaeroides* matings by plating 0.1 ml of donor and 0.1 ml of recipient on dry LB or Sistrom A agar plates, incubating at 30°C for 9 to 16 h, resuspending the cells in broth, and plating on the appropriate selective agar medium.

Construction of plasmids and Mu dl(Ap *lac* Km). Strain UC4200 was infected with a heat-induced lysate from strain MA1103 and plated on McConkey lactose agar medium supplemented with 50 μg of sodium ampicillin per ml. Ap^r survivors were streak purified. Separate cultures of UC4200::Mu dl(Ap *lac*) were grown at 37°C for 12 h to partially induce Mu dl(Ap *lac*). The cultures were washed and suspended in M9 medium without Ca^{2+} or Mg^{2+}, and 0.3 ml was mixed with 0.3 ml of a similarly prepared culture of UC4288. After 2.5 h, the cell mixture was plated on M9-glucose agar supplemented with proline, ampicillin, and trimethoprim. Exconjugants from independent matings were tested for cotransfer of Ap^r and Tp^r and used in subsequent matings to *R. sphaeroides*.

To generate a Mu dl(Ap *lac*) bacteriophage containing a

[a] Wild-type *R. sphaeroides* 2.4.1. does not grow on glucose and β-galactosidase activity is not detectable.

[b] The numbers in parentheses after the LacZ designations represent the specific activity of β-galactosidase as defined in the text.

[c] Pho, Photosynthetic phenotype. Designations is parentheses: B800-850$^-$, absorption peaks at 800 and 850 nm missing; B875$^-$, absorption peak at 875 nm missing; B800-850/B875, ratio of peaks is altered.

[d] We are unable to explain the reversion to wild type photosynthetic phenotype in strains T19 and T20. It should be noted that several other photosynthetic mutants did not have changes in their photosynthetic phenotype when cured of R751::Mu dl(Ap *lac*) or R751::Mu dl(Ap *lac* Km).

[e] Ampicillin resistance is not expressed in *R. sphaeroides*.

[f] All of the bacteriophages have a temperature-sensitive repressor protein. Mu dl(Ap *lac*) and Mu dl(Ap *lac* Km) do not produce viable phages.

[g] A nontransposable *aphA* locus (derived from Tn*5*) is located between the *B* and *C* genes at the *C* end of Mu.

Km^r marker, we performed the following manipulations. Strain MC4100::Mu d1(Ap *lac*) was superinfected with Mu *cts*62 pf7701Δ445-3, and one Ap^r Km^r survivor (MN1) that was both temperature sensitive and unable to produce viable bacteriophage was streak purified and used in subsequent experiments. UC3014 was crossed with MN1 to introduce a nondefective Mu. The heat-induced lysate from MN1(pGM14) was used to infect MC4100, and either Ap^r or Km^r was selected for. We found 121 of 121 of the Ap^r transductants to also be Km^r and 62 of 62 Km^r transductants to be Ap^r, indicating that we had isolated a true recombinant bacteriophage carrying both Ap^r and Km^r markers. Infection of MC4100 with presumptive Mu d1(Ap *lac* Km) yielded lysogens with a spectrum of Lac phenotypes. Several recombinant R751::Mu d1(Ap *lac* Km) plasmids were constructed as described above.

Isolation and manipulation of DNA. Plasmid DNA was isolated by the method of Humphreys et al. (14) followed by CsCl centrifugation or by the rapid method of Ish-Horowicz and Burke (15). Rapid preparations of chromosomal DNA were prepared as described previously (5), whereas DNA used for restriction digest was subjected to a more extensive treatment of organic solvent extraction and winding out of ethanol (31). Plasmid DNA was separated on 0.6% agarose gels at 150 V for 11.5 h. Restriction digests were separated on 1% agarose gels by using 100 V for 6 h. Gels were run submerged in 89 mM Tris-borate buffer (pH 8.3) (20) in a Bethesda Research Laboratories model HO gel electrophoresis system.

DNA was labeled by nick translation (29) with a Bethesda Research Laboratories nick translation kit and [α-^{32}P]dCTP (800 Ci/mmol) from New England Nuclear Corp. or Amersham Corp. The DNA probe was labeled to at least 10^7 cpm/μg. DNA to be used as a probe was isolated from agarose gels as described previously (38). Plasmids to be used for restriction digest were isolated in a 5 to 20% sucrose gradient with 10 μg of ethidium bromide per ml included in the gradient. Centrifugation was carried out in an SW41 rotor at 30,000 rpm for 1.5 h at 20°C.

DNA was transferred from agarose gels to Gene Screen (New England Nuclear) via capillary action by the method of Southern (36). Gels were photographed with UV fluorescence (exposure time, 2.5 min) and treated with 0.25 N HCl for 5 min before blotting. All other procedures for blotting and hybridizations were as recommended by the Gene Screen manual (hybridization method III). After hybridization with a radioactive probe, Southern blots were exposed to X-ray film (Kodak XAR-5) with an intensifying screen (Cronex Hi-plus or Lightning-plus) for various periods of time. The hybridization conditions were 20°C below the effective melting temperature of *R. sphaeroides* DNA.

Other methods. β-Galactosidase assays were performed on whole cells after permeabilization with sodium deoxycholate (100 μg/ml) and hexadecyltrimethylammonium bromide (200 μg/ml) for 25 min. *ortho*-Nitrophenyl-β-D-galactopyranoside (ONPG) was used as the substrate, and enzyme units were expressed as nanomoles of ONPG cleaved per minute per milligram of protein. The extinction coefficient of *ortho*-nitrophenol was taken to be 7,500 as determined by Pardee et al. (25). Protein concentrations were determined by a microbiuret assay (23) after solubilizing cells for at least 1 h in 1.5 N NaOH.

McConkey lactose agar plates (Difco Laboratories) were used to detect β-galactosidase activity in *E. coli*, and Sistrom A agar plates supplemented with 40 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) per ml were used to detect β-galactosidase activity in *R. sphaeroides*. When using X-Gal, it was essential to mix 2× Sistrom medium and 2× agar solutions after autoclaving to yield clear agar medium.

Chromatophores (isolated ICM vesicles) were prepared as described previously (11). Visible and infrared spectra were performed on crude preparations of chromatophores (13,000 × *g* supernatant of sonicated cells) by using a Cary 14 spectrophotometer.

Chemicals and enzymes. All antibiotics were purchased from Sigma Chemical Co. Sodium ampicillin was used at 50 μg/ml, tetracycline HCl at 10 μg/ml, kanamycin SO_4 at 20 μg/ml; and trimethoprim at 50 μg/ml for use with *R. sphaeroides* and 100 μg/ml for use in *E. coli* minimal media. X-Gal was purchased from Bachem. Restriction enzymes were obtained from Boehringer Mannheim and Bethesda Research Laboratories and used as recommended previously (5).

RESULTS

Mating R751::Mu d1(Ap *lac*) and R751::Mu d1(Ap *lac* Km) plasmids to *R. sphaeroides*. Since we were unable to infect *R. sphaeroides* with bacteriophage Mu or derivatives of Mu, we chose to introduce Mu d1(Ap *lac*) via the P-incompatibility group plasmid R751. After mating of *R. sphaeroides* by *E. coli*, selection for exconjugants was made on Sistrom-plus-trimethoprim agar medium; exconjugants were then scored for β-galactosidase activity by transferring colonies to Sistrom agar medium supplemented with X-Gal. Normally *R. sphaeroides* is Lac^- (24). We found that >98% of the exconjugants of FN22 × *R. sphaeroides* 2.4.1 had low levels of β-galactosidase (see Table 2), suggesting that in the construction of R751::Mu d1(Ap *lac*), Mu d1(Ap *lac*) was linked to a weak plasmid transcriptional promoter. However, we observed that ca. 1% or less of the exconjugants had higher β-galactosidase activity (deep blue on X-Gal agar

TABLE 2. Characteristics of *E. coli* [R751::Mu d1(Ap *lac*)] × *R. sphaeroides* and *E. coli* [R751::Mu d1(Ap *lac* Km)] × *R. sphaeroides* exconjugants

Donor strain (β-galactosidase sp act)[a]	Selected marker for exconjugants	Unselected marker(s)	β-Galactosidase sp act of >98% of *R. sphaeroides* exconjugants[a]	Appearance of >98% of *R. sphaeroides* exconjugants on X-gal plates	Frequency of deep blue exconjugants (per total exconjugant population)
FN22 (19)	Tp^r	β-Galactosidase	10	Pale blue	1.0×10^{-2}
FN23 (35)	Tp^r	β-Galactosidase	31	Light blue	
FN52 (15)	Km^r	Tp^r, β-galactosidase	75	Deep blue	
FN56 (4)	Km^r	Tp^r, β-galactosidase	10	Pale blue	5×10^{-3}
FN59 (4)	Km^r	Tp^r, β-galactosidase	3.2	Pale blue	1.5×10^{-2}

[a] Specific activity of β-galactosidase expressed as nanomoles of *o*-nitrophenyl-β-D-galactopyranoside cleaved per minute per milligram of protein.

TABLE 3. Phenotype of *R. sphaeroides* mutants cured of R751::Mu dl(Ap *lac*) or R751::Mu dl(Ap *lac* Km) with pRK290

Strain	Phenotype[a]
L14	Tp^r β-Gal$^+$ Pho$^-$
L19	Tp^r β-Gal$^+$ Pho$^-$
L20	Tp^r β-Gal$^+$ Pho$^-$
L33	Tp^r Km^r β-Gal$^+$ Pho$^-$
T14	Tc^r Tp^s β-Gal$^-$ Pho$^-$
T19	Tc^r Tp^s β-Gal$^-$ Pho$^+$
T20	Tc^r Tp^s β-Gal$^-$ Pho$^+$
T33	Tc^r Tp^s Km^s β-Gal$^-$ Pho$^-$

[a] Pho$^-$ indicates that a strain is either unable to grow photosynthetically or has altered pigments. Spectra and β-galactosidase assays were performed on all strains, except L33, in which case β-galactosidase and Pho phenotypes were assayed by inspection of colonies on Sistrom or Sistrom plus X-gal agar medium.

medium) and that a high number of photosynthetically altered clones were present among the exconjugants (wild-type *R. sphaeroides* is red on agar medium, and color mutants can be discerned easily). Occasionally, exconjugants were found that had no β-galactosidase activity. The results with FN23 as donor were essentially identical to those of FN22 × *R. sphaeroides* except that the "normal" exconjugants (>98%) produced somewhat higher β-galactosidase levels.

Since the β-lactamase marker of Mu dl(Ap *lac*) is not expressed in *R. sphaeroides* (22) we were unable to trace Mu dl(Ap *lac*) apart from its LacZ phenotype. Accordingly we introduced a Km^r marker into Mu dl(Ap *lac*) as described above. The Km^r marker is derived from Tn*5* but is nontransposable (M. Howe, personal communication). The presence of the Km^r marker allowed us to assay for the presence of Mu dl(Ap *lac*) in the absence of selective pressure for R751.

The mobilization of independent R751::Mu dl(Ap *lac* Km) plasmids into *R. sphaeroides* gave essentially the same results as discussed above for R751::Mu dl(Ap *lac*) plasmids (see Table 2). Most exconjugants expressed low β-galactosidase levels and about 1% or less expressed elevated levels. When FN56 and FN59 were used as donors, we observed a >99% cotransfer of Tp^r and Km^r regardless of which marker was selected. It should be noted that the fraction of photosynthesis-deficient exconjugants per total exconjugants population varied considerably from cross to cross but averaged about 10^{-5}. The β-galactosidase levels of the *R. sphaeroides* exconjugants were approximately the same levels as their *E. coli* donors except for the exconjugants of the FN52 × *R. sphaeroides*, which had fivefold higher β-galactosidase levels than FN52.

Because we found photosynthesis-deficient mutants among the exconjugants which also possessed elevated β-galactosidase activity, we reasoned that these represented a potential fusion of Mu dl(Ap *lac*) to photosynthetic operons. A number of these exconjugant clones were purified and examined for photosynthetic phenotype, β-galactosidase levels, plasmid complement, and other properties. To simplify the presentation of the data, we have provided information about three strains L14, L19, and L20 (and sometimes include strain L33).

Curing of R751::Mu dl(Ap *lac*) from *R. sphaeroides* strains. If Mu dl(Ap *lac*) had transposed to endogenous DNA of *R. sphaeroides* then Lac expression should be independent of the presence or absence of R751. We were able to cure R751 from *R. sphaeroides* by the introduction of another P-group plasmid, pRK290. Since pRK290 is not self-transmissable, we used pRK2013, which is unable to maintain itself in *R. sphaeroides* as a mobilizing plasmid (see Table 3). In every instance both Tp^r and β-galactosidase activity (and Km^r where appropriate) were eliminated by the introduction of pRK290. The linkage of Tp^r and the β-galactosidase positive phenotype suggested that any copies of Mu dl(Ap *lac*) in the cell reside on R751.

The direct physical confirmation of the presence or absence of R751::Mu dl(Ap *lac*) was demonstrated by agarose gel electrophoresis of isolated plasmids. *R. sphaeroides* 2.4.1 has five indigenous plasmid species of sizes 114, 104, 99.5, 99, and 42 kb; the 99- and 99.5-kb plasmids migrate as a doublet on agarose gels (Fig. 1, lane 6). The plasmid complement of strains L14, L19, and L20 was found to be altered from that of wild-type *R. sphaeroides*, and these plasmid profiles are discussed below. In each of these strains, however, one plasmid species was excluded by the introduc-

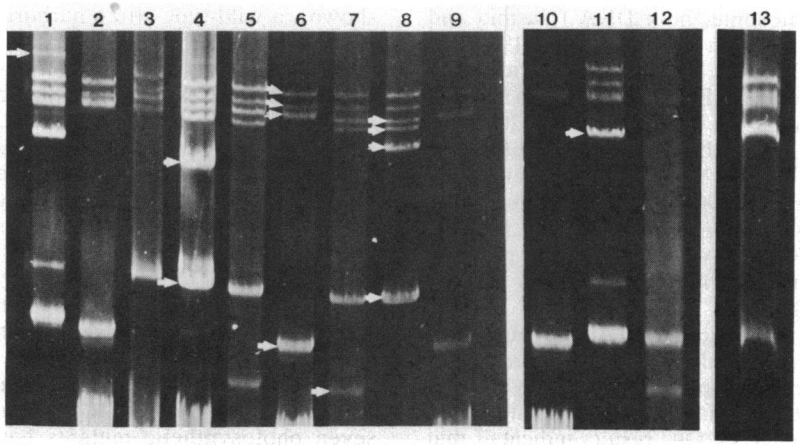

FIG. 1. Agarose gel analysis of plasmids isolated from photosynthetic mutants harboring R751::Mu dl(Ap *lac*). Lane 1, L20, arrow points to the 125-kb plasmid; lane 2, strain 2.4.1; lane 3, L19-1; lane 4, L19, top arrow points to the 82-kb plasmid, bottom arrow points to the 50-kb plasmid; lane 5, T19; lane 6, strain 2.4.1, arrows point to (top to bottom) the 114-kb plasmid, the 104-kb plasmid, doublet plasmid band at 99 kb, and the 42-kb plasmid (pRS2.4.1e); lane 7, T14, Arrow points to open circular form of pRK290; lane 8, L14, arrows point to (top to bottom) the 99-kb, 96-kb, 88-kb [R751::Mu dl(Ap *lac*)], and 50-kb plasmids; lane 9, strain 2.4.1; lane 10, strain 2.4.1; lane 11, L20, arrow points to R751::Mu dl(Ap *lac*) plasmid; lane 12, T20; lane 13, L1.

tion of pRK290; such strains are designated by the prefix T. In strain L14 the 88-kb plasmid (Fig. 1, lane 8) that comigrated with R751::Mu d1(Ap *lac*) was eliminated as was the 87.5-kb plasmid species in L20 (Fig. 1, lane 11). Strain L19 possesses a plasmid of 82 kb (see Fig. 1, lane 4) which is excluded by pRK290. Also, strain T19-1, a spontaneous β-galactosidase negative, Tp^s segregant of L19 similarly lacks the 82-kb plasmid (lane 5). Since the Tp^r and β-galactosidase markers in L19 were readily lost in the absence of Tp and since Tp^r could not be transferred to other strains, we assumed that a deletion of R751::Mu d1(Ap *lac*) in L19 generated the 82-kb plasmid and eliminated certain plasmid maintenance and transfer functions.

β-Galactosidase levels of R751::Mu d1(Ap *lac*) plasmids in other genetic backgrounds. A third approach to assessing the linkage of presumed plasmid markers is to transfer the plasmid to another strain and score the phenotype of the exconjugants. Plasmids were transferred from strains L14, L20, and L33 to another *R. sphaeroides* strain as well as to an *E. coli* (Mu *c*ts62 Δ*lac*) background, and we found the same β-galactosidase levels in the exconjugants as in the parent strains (data not shown). This indicated that β-galactosidase expression was controlled by plasmid promoters. We were unable to conjugate or transform the plasmid in L19 to other strains.

Southern blot analysis of R751::Mu d1(Ap *lac*) plasmids. Besides using in vivo genetic tests, we were in a position to ascertain the location of Mu d1(Ap *lac*) within the *R. sphaeroides* strains under study. We therefore examined *R. sphaeroides* strains L14, L19, L20, T14, T19, T20, and 2.4.1. by Southern blot hybridization and spot hybridization, with pMB041 and R751::Mu d1(Ap *lac*) as probes. Plasmid pMB041 is a pBR322 derivative which contains an insert of a *Hin*dIII-*Pst*I fragment of the S end of Mu d1(Ap *lac*) and includes portions of the β-lactamase *lac* operon and some Mu sequences (M. B. O'Connor and M. Malamy, personal communication). This plasmid has no sequence homology to pRK290. pMB041 hybridized to the R751::Mu d1(Ap *lac*) plasmid bands in each strain but not to any other plasmid bands (Fig. 2). In strains L20 (lane 2) and L19 (lane 4), the presumptive deletions in the R751::Mu d1(Ap *lac*) plasmids make them migrate ahead of the parent R751::Mu d1(Ap *lac*) plasmid (lanes 3, 5, and 8).

To examine whether Mu d1(Ap *lac*) transposed to a site on the *R. sphaeroides* chromosome, bulk DNA (plasmid and chromosome) preparations from strains L14, L19, L20, 2.4.1, T14, T19, and T20 were spotted on hybridization substrate and hybridized with pMB041. If Mu d1(Ap *lac*) had transposed to a chromosomal site in L14, L19, or L20 then a copy of Mu d1(Ap *lac*) would be expected to remain in strains T14, T19, and T20 [which had been cured of R751::Mu d1(Ap *lac*) with pRK290]. Figure 2C shows 0 and 10-fold dilutions of DNA preparations from strains L14, L19, and L20 hybridized to pMB041, whereas identical preparations of DNA from strain T14, T19, T20, and 2.4.1 showed no hybridization to pMB041.

Conceivably, segments of R751::Mu d1(Ap *lac*) outside of the S end of Mu d1(Ap *lac*) could have transposed to the endogenote DNA. To test this possibility, we hybridized R751::Mu d1(Ap *lac*) to *Eco*RI-cut bulk DNA isolated from strains L14, L19, L20, and L33. Our results indicated that there are no R751::Mu d1(Ap *lac*) sequences physically associated with the endogenote DNA since all of the hybridizing bands in L14, L19, and L20 align with those of pure R751::Mu d1(Ap *lac*) (data not shown).

Photosynthetic mutants isolated after the introduction of R751::Mu d1(Ap *lac*) and R751::Mu d1(Ap *lac* Km) into *R. sphaeroides*. Concomitant with the introduction of both R751::Mu d1(Ap *lac*) and R751::Mu d1(Ap *lac* Km) into *R. sphaeroides*, numerous spectrally distinct mutants were generated (Fig. 3). The presence of R751::Mu d1(Ap *lac*) and high β-galactosidase levels were by themselves not sufficient to alter the absorption spectrum of *R. sphaeroides* as indicated by the spectrum of strain L1 (Fig. 3, panel B; data not shown), a wild-type strain harboring R751::Mu d1(Ap *lac*). Alterations in the absorption profile can be the result of the loss, changes in, or altered amounts of different pigment-protein complexes. Photosynthetic mutants arising as the result of the introduction of Mu d1(Ap *lac*) included mutants with alterations in various absorption maxima (Fig. 3), and in some cases the alterations could be attributed to a loss of a specific polypeptide (data not shown).

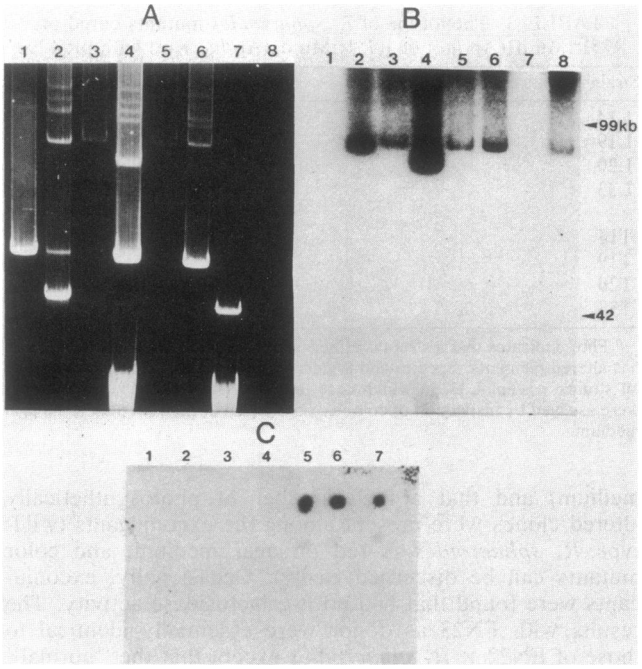

FIG. 2. Agarose gel electrophoresis and DNA-DNA hybridization. (A) Agarose gel (0.6%) electrophoresis of plasmids. (B) Autoradiogram of Southern blot. Probe is ^{32}P-labeled pMBO41. Lane 1, Plasmid R751; lane 2, L20; lane 3, plasmid pUI2; lane 4, L19; lane 5, plasmid pUI2; lane 6, L14; lane 7, strain 2.4.1; lane 8, plasmid pUI2. (C) Dot hybridization with pMBO41 as probe. Bulk DNA was isolated by a rapid method, and 0-, 10-, and 100-fold dilutions of DNA preparations were spotted on Gene Screen filter paper. Lane 1, T20; lane 2, T19; lane 3, T14; lane 4, strain 2.4.1; lane 5, L20; lane 6, L19; lane 7, L14.

Physiological studies in which we measured β-galactosidase activity of cultures shifted from growth under high oxygen tension to growth under low oxygen tension (to induce photosynthetic development) failed to produce increases in β-galactosidase activity (data not shown) in strains L14, L19, and L20.

Plasmid profiles of photosynthetic mutants harboring R751::Mu d1(Ap *lac*) and R751::Mu d1(Ap *lac* Km). Seven of seven photosynthetic mutants harboring R751::Mu d1(Ap *lac*) were found to have alterations in their indigenous plasmid profiles as typified by strains L14, L19, and L20. Strains L14 and L19 were missing pRS2.4.1.e (42-kb plasmid), and a new plasmid species of 50 kb was present (Fig. 1, lanes 4 and 8). Further, there is no doublet band at the

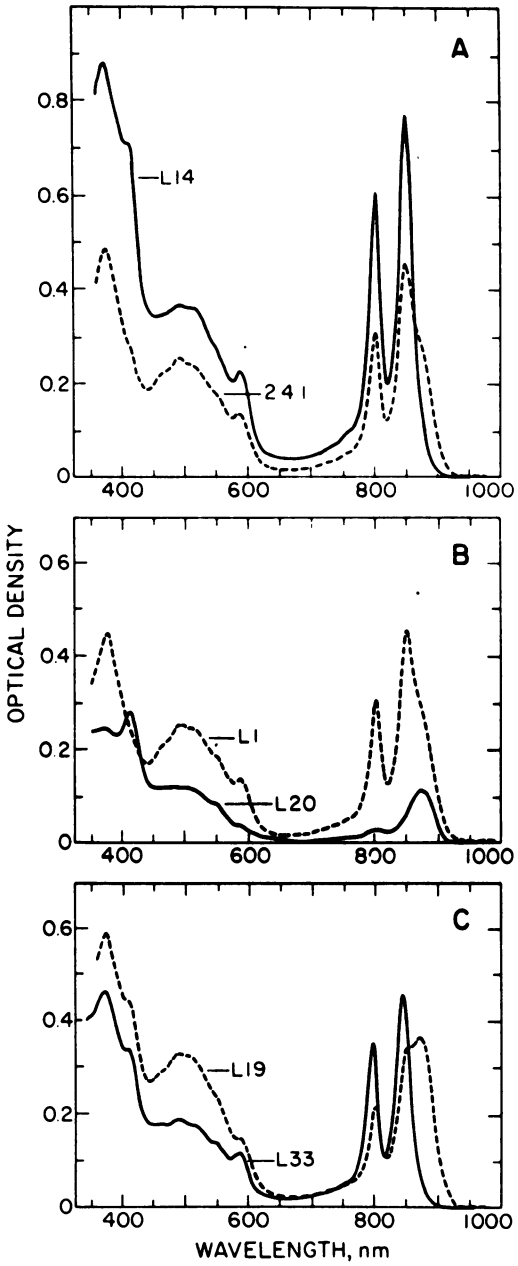

FIG. 3. Spectra of crude chromatophore preparations isolated from *R. sphaeroides* strains. (A) Wild-type strain 2.4.1 and mutant L14. (B) Strains L1 and L20. Strain L1 is a wild-type strain harboring plasmid pUI2. (C) Strains L19 and L33. All strains were grown at 2.7% O_2 to induce the formation of ICM.

position of 99 kb in strains L14 and L19, and instead a new plasmid band is seen at ca. 96 kb (Fig. 1, lane 8) together with a single band at 99 kb. Strain L20 has plasmids of ca. 43 and 51 kb and is missing pRS2.4.1.e (Fig. 1, lane 1). Surprisingly, eight of eight photosynthetic mutants induced by the introduction of R751::Mu dl(Ap *lac* Km) did not have rearrangements of their indigenous plasmids except for strain L33, which had a 43-kb plasmid rather than a 42-kb plasmid. The introduction of R751 into *R. sphaeroides* did not produce plasmid rearrangements.

Southern blot analysis of rearranged *R. sphaeroides* plasmids. Since the plasmid profile of several photosynthesis-negative exconjugants showed the absence of pRS2.4.1e and the appearance of a new plasmid of 50 kb, we reasoned that these two plasmid species may be related. pRS2.4.1e from strain 2.4.1 and the 50-kb plasmid from strain L19 were purified and labeled with ^{32}P by nick translation and were used to probe Southern blots of the plasmids isolated from strains L14, L19, L20, and 2.4.1 (Fig. 4). We found that both pRS2.4.1e and the 50-kb plasmid hybridized to the same plasmid bands; they both hybridized to pRS2.4.1.e, the 50-kb plasmid, the doublet in strain 2.4.1 at 99 kb, and to the band at 96 kb in strains L14 and L19 (seen best in Fig. 4A and B, lanes 3, 4, 5, and 6). It is important to note that in strains L14 and L19 there was hybridization to the 96-kb plasmid and no hybridization to the 99-kb plasmid. This suggested that one of the plasmids in the 99-kb doublet in the wild-type strain 2.4.1 hybridizes to pRS2.4.1e and the 50-kb plasmid and that in strains L14 and L19, the same 99-kb plasmid presumably lost sequences to become the 96-kb plasmid. Also, there is a hybridizing band at 125 kb which can be seen in strain L20 (Fig. 4A, lane 1). A hybridizing band at 125 kb in strain 2.4.1 can be seen after prolonged

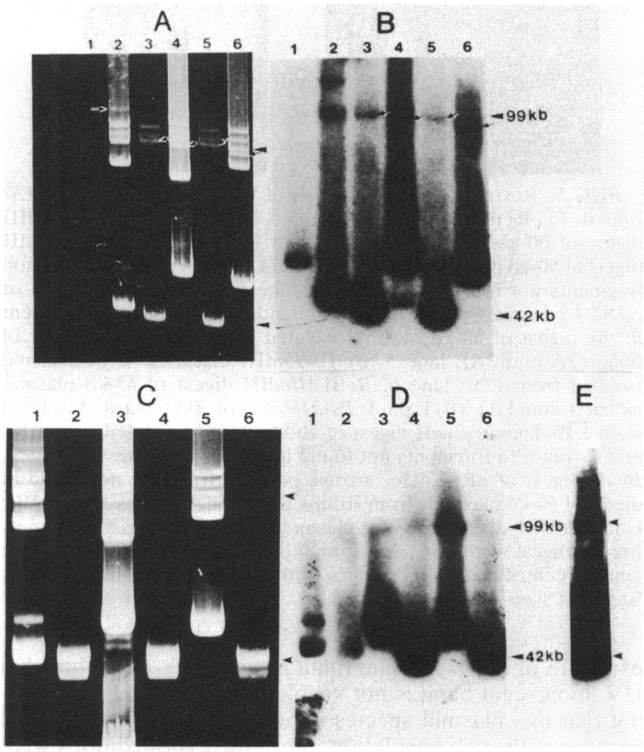

FIG. 4. Agarose gel electrophoresis and DNA-DNA hybridization of plasmids from *R. sphaeroides* strains. (A and C) Agarose gel (0.6%) electrophoresis of plasmid DNA visualized by UV fluorescence of ethidium bromide-stained gel. (B and D) Autoradiogram of Southern blots of the gels in (A) and (C), respectively. Probe used in (B) was ^{32}P-labeled 50-kb plasmid isolated from strain L19. Probe used in (D) was ^{32}P-labeled pRS2.4.1e. (A and B) Lane 1, 50-kb plasmid isolated from strain L19; lane 2, L20; lane 3, strain 2.4.1; lane 4, L19; lane 5, strain 2.4.1; lane 6, L14. Note that the plasmid bands binding the probe in lanes 4 and 6 migrated faster than the hybridizing bands in lane 3 and 5. In lane 2, the light band at 125-kb binds the probe. (C and D) Lane 1, L20; lane 2, strain 2.4.1; lane 3, L19; lane 4, strain 2.4.1; lane 5, L14; lane 6, strain 2.4.1. Arrows at sides of panels indicate the 99- and 42-kb region and help align the agarose gels and the Southern blots. (E) A longer exposure of lane 4 in (D).

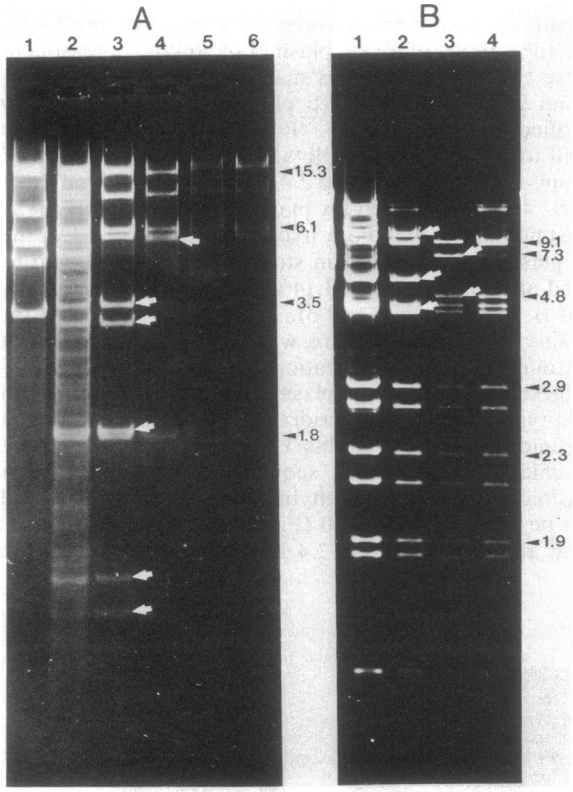

FIG. 5. Restriction digest analysis of 50- and 43-kb plasmids. (A) Lane 1, *Eco*RI digest of bacteriophage λ DNA; lane 2, *Bgl*II-*Hin*dIII digest of 50-kb plasmid isolated from L19; lane 3, *Bgl*II-*Hin*dIII digest of 50-kb plasmid isolated from L14, arrows point to restriction fragments not found in pRS2.4.1e; lane 4, *Bgl*II-*Hin*dIII digest of pRS2.4.1e, arrow points to bottom band in doublet which is absent in the digest of 50-kb plasmid isolated from strains L19 and L14 (lanes 2A and 3A); lane 5, *Bgl*II-*Hin*dIII digest of 43-kb plasmid isolated from L20; lane 6, *Bgl*II-*Hin*dIII digest of 43-kb plasmid isolated from L33. (B) Lane 1, *Pvu*II digest of 50-kb plasmid isolated from L19; lane 2, *Pvu*II digest of 50-kb plasmid isolated from L14, arrows point to fragments not found in pRS2.4.1e (lane 3B); lane 3, *Pvu*II digest of pRS2.4.1e, arrows point to fragment not found in digest of 50-kb plasmids from strains L19 and L14 (lanes 1B and 2B); lane 4, *Pvu*II digest of 43-kb plasmid isolated from L20. Plasmids used in digest were separated from other plasmid species by sucrose density centrifugation. Numbers at the side of the panels indicate fragment sizes in kb. (10).

exposure of the X-ray film (data not shown), even though a UV fluorescent band is not visible on agarose gels, suggesting that this plasmid species is normally present in a small fraction of the cell population. Since the hybridizations were performed under high stringency conditions, there must be extensive DNA sequence homology among the cross-reactive plasmid species. It is unlikely that the 99- and 96-kb plasmid species are open circular plasmid forms of the 42- and 50-kb plasmids, respectively, since one would expect the open circular form of a 50-kb plasmid to migrate more slowly than the open circular form of a 42-kb plasmid. Precisely the opposite situation is found: strain 2.4.1 has 42- and 99-kb plasmids which reveal DNA homology and strains L14 and L29 have 50- and 96-kb plasmids which possess homology.

Restriction digest analysis of 43- and 50-kb plasmids. Plasmids of 50, 42, and 43 kb were isolated by sucrose gradient rate zonal centrifugation from strains L14 and L19, 2.4.1., and L20, respectively, and subjected to two endonuclease digestions (Fig. 5). Both a *Pvu*II digest and a double *Bgl*II-*Hin*dIII digest indicated that these three plasmid species have extensive physical identity. The *Pvu*II digest showed that the 7.3- and 4.8-kb fragments present in pRS2.4.1e were missing in the 50-kb plasmids of strains L14 and L19 and that new fragments of 10, 5.6, and 4.4 kb were present, giving the total amount of additional DNA (above 42 kb) as 7.8 kb. The *Bgl*II-*Hin*dIII digest of the 50-kb plasmids of L14 and L19 showed one of the pRS2.4.1e fragments of 5.6 kb to be missing (at doublet; Fig. 5A, lane 4) as well as the presence of additional fragment bands for a total of 7 kb of additional DNA. Although contaminating DNA was present in the L19 plasmid preparation, it was apparent that the same pRS2.4.1e fragments were missing from the 50-kb plasmids of L14 and L19, indicating that the DNA alteration occurred in the same region in both strains, which are independent isolates. The fact that two *Pvu*II restriction fragments derived from pRS2.4.1e were altered in the 50-kb plasmids indicated that either two independent insertions occurred in two unique *Pvu*II fragments of pRS2.4.1e or that a substitution reaction occurred which spanned a *Pvu*II restriction site of pRS2.4.1e. Conceivably, one insertion could have occurred precisely at a *Pvu*II site of pRS2.4.1e. The *Bgl*II-*Hin*dIII digests of the 43-kb plasmids from L20 and L33 were identical, the largest fragment being ca. 1 kb larger than the corresponding fragment in pRS2.4.1e.

Southern blot analysis of restriction digests of large (99 to 114 kb) plasmids. Strain CU1022 lost pRS2.4.1e but retained the plasmids of 99 to 114 kb. Using restriction digests of both plasmid DNA and bulk DNA (chromosomal plus plasmid) from strains CU1022 and 2.4.1, we were able to determine whether there were DNA sequences exogenous to pRS2.4.1e that combined with pRS2.4.1e to form the 50-kb plasmid of strain L19. We were further able to test whether the origin of any exogenous DNA was plasmid or chromosomal.

In Fig. 6 we see that when pRS2.4.1e was used as probe against *Pvu*II-digested bulk and plasmid DNA from strains CU1022 and 2.4.1, hybridizing bands were found in the CU1022 digest which aligned precisely with pRS2.4.1e restriction fragments present in the 2.4.1 digest. Five of the restriction fragments of pRS2.4.1e were not found in the CU1022 digest (missing bands are indicated by small arrows in Fig. 6B). In addition, one (at 25 kb) and possibly three (at 7.9 and 2.0 kb) hybridizing bands appeared in the 2.4.1 and CU1022 *Pvu*II digests which did not correspond to any of the *Puv*II fragments of pRS2.4.1e (Fig. 6B). Consistent with these observations we found that in *Eco*RI digests of the plasmid DNA, the single 42-kb digestion product of pRS2.4.1e was missing in CU1022. In addition, both 2.4.1 and CU1022 had additional hybridizing fragments at ca. 25 and 15 kb in their *Eco*RI digest (Fig. 6B, lane 7). These results coupled with the results presented in Fig. 4 indicated that in strains CU1022 and 2.4.1. there is a large stretch of DNA sequences resident in one of the large plasmids that is homologous with pRS2.4.1e sequences. Moreover, since all of the hybridizing sequences found in bulk DNA are also found in the plasmid DNA we conclude that no pRS2.4.1e sequences are present in the chromosomal DNA. The discovery that there is extensive homology between pRS2.4.1e and one of the large plasmids has implications for the mechanism of the plasmid rearrangements described above.

When the 50-kb plasmid was used as a probe to an identical Southern blot as in Fig. 6 (strains 2.4.1 and CU1022), we found an additional hybridizing band in both

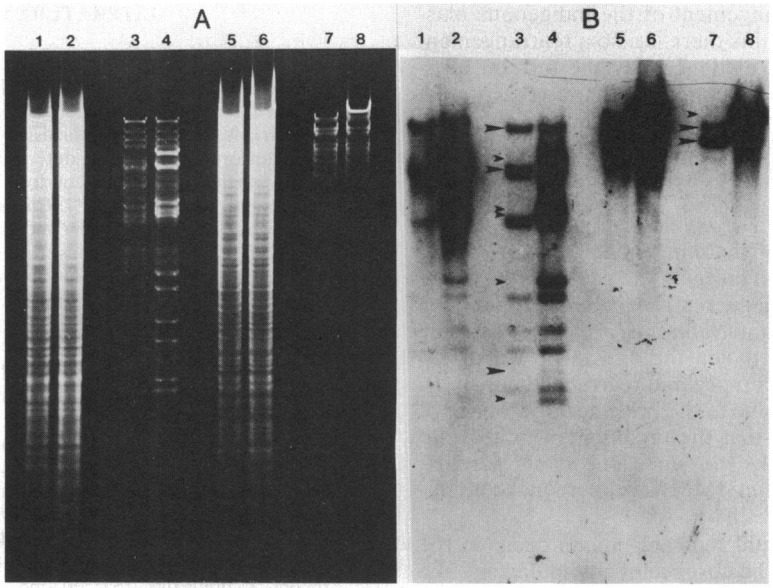

FIG. 6. Agarose gel electrophoresis and DNA-DNA hybridization of restriction digest of bulk and plasmid DNA. (A) Agarose gel electrophoresis of digested DNA. (B) Autoradiogram of Southern blot of gel in (A). Lanes 1 and 2, *Puv*II digest of bulk DNA isolated from strains CU1022 and 2.4.1, respectively; lanes 3 and 4, *Pvu*II digest of plasmid DNA isolated from strains CU1022 and 2.4.1, respectively; lanes 5 and 6, *Eco*RI digest of bulk DNA isolated from strains CU1022 and 2.4.1, respectively; lanes 7 and 8, *Eco*RI digest of plasmid DNA isolated from strains CU1022 and 2.4.1, respectively. Plasmid pRS2.4.1e labeled with ^{32}P was used as probe. The small arrows point to hybridizing fragments that are present in strain 2.4.1 but are not present in CU1022. The large arrows point to hybridizing fragments that cannot be attributed to a pRS2.4.1e restriction fragment. Some of the hybridizing bands can be seen clearly only after longer exposures.

the *Pvu*II and *Eco*RI digest of the 2.4.1 and CU1022 DNA (data not shown). These data indicated that some DNA sequences extraneous to pRS2.4.1.e are present in the 50-kb plasmid of strain L19. This suggested that whatever the mechanism involved in the formation of the 50-kb plasmid it does not simply involve recombination between completely homologous DNAs. Similarly, no hybridizing bands were found in bulk DNA that were not also present in plasmid DNA.

By summation of the hybridizing bands that are known to be *Pvu*II restriction fragments of pRS2.4.1e, we found a minimum of 19 kb of pRS2.4.1e sequences present in the CU1022 plasmid DNA. There were two or possibly three DNA fragments in the CU1022 and 2.4.1 *Pvu*II digest of plasmid DNA which hybridized to pRS2.4.1e sequences but which were known not to be *Pvu*II fragments of pRS2.4.1e (Fig. 6). We cannot know how much of the pRS2.4.1e sequences were present in these bands but if we assume that the two largest pRS2.4.1e *Pvu*II fragments which were absent from CU1022 plasmid DNA (9.1 and 4.8 kb) were incorporated into those bands then we can place the maximum amount of pRS2.4.1e sequences present in CU1022 DNA as 33 kb. Analysis of Southern hybridization blots of *Bgl*II and *Sst*I restriction digests supported the estimated size range of pRS2.4.1e sequences present in CU1022 plasmid DNA (data not shown) but did not allow a more accurate estimation.

DISCUSSION

Genomic rearrangements are widespread, particularly among procaryotes, where it is often found that the genes affected by the rearrangements are essential to the survival of the organism. For example, the flagellar and pilus proteins of *Salmonella* and *Neisseria* spp., respectively, are thought to be important in attachment of these pathogens in their host, and both surface components are subject to switching to another form as a result of DNA rearrangements (18, 19, 34, 39). Similarly, the form of the tail fiber proteins of bacteriophages Mu and P1 are controlled by an inverting DNA element (2, 4, 16). In *Rhizobium* the inactivation of nitrogen-fixation genes can be effected by plasmid rearrangements (7) or the specific insertion of an insertion element (30).

The coincidence of appearance in *R. sphaeroides* of photosynthetic mutants accompanied by plasmid rearrangements strongly suggests the involvement of such rearrangements in generating those phenotypes. The data presented here unambiguously demonstrate that these plasmid rearrangements involve pRS2.4.1e and a 99-kb plasmid in the wild-type, resulting in the formation of a 50-kb plasmid and a 96-kb plasmid in some mutants. Hybridization of the 50-kb plasmid to restriction enzyme-digested bulk and plasmid DNA indicated that no chromosomal DNA sequences were present on the 50-kb plasmid. Conceivably, the 125-kb plasmid is a cointegrate structure between sequences of pRS2.4.1e and one of the 99-kb plasmids which resolves itself into the 50-kb plasmid and a 96-kb plasmid. Such cointegrate formation between plasmids with extensive homology has been demonstrated recently (26).

The data presented in this work confirm and expand the observations of Saunders and co-workers (33) who found pRS2.4.1e replaced by a 50-kb plasmid in a photosynthetic mutant. Their observation that pRS2.4.1e, the 50-kb plasmid, and a 99-kb plasmid all had a buoyant density of 1.717 g/cm^3 (58% G+C), whereas the 114-kb plasmid had a buoyant density (1.724 g/cm^3) and G+C content (65%) representative of chromosomal DNA adds further weight to the idea of a special relatedness of the plasmids involved in the rearrangements.

Given the specificity of the plasmid rearrangements, how is the introduction of R751::Mu dl(Ap *lac*) related to these events? We demonstrated that Mu DNA need not be physi-

cally involved in the rearrangement of the indigenous plasmids. However in some strains there is also a rearrangement of R751::Mu dl(Ap *lac*) as evidenced by the turn-on of β-galactosidase in some strains and the obvious deletion of R751::Mu dl(Ap *lac*) in strain L19. We believe, therefore, that two influences of Mu are in effect in certain strains, the integration and replication function of Mu effecting well-documented Mu-induced DNA rearrangements (8) of R751:Mu dl(Ap *lac*) and a *trans*-acting influence of Mu effecting a rearrangement of the indigenous plasmids.

The *gin* gene of Mu is thought to be the causal agent of the *trans*-acting induction of gene rearrangement such as the inversion of the *PD* element in *Salmonella* species (18). The differences we observed between the effects of Mu dl(Ap *lac*) and Mu dl(Ap *lac* Km) on plasmid rearrangements in *R. sphaeroides* may in fact be due to the presence or absence of the *gin* gene. It is possible that the recombinant Mu dl(Ap *lac* Km) bacteriophage lacks *gin* since one of its parents, Mu *cts*62 pf7701Δ445-3, in *gin*$^-$ (M. Howe, personal communication).

We also found that plasmid rearrangements occur in the absence of Mu and even in the absence of any mutagens. We have isolated photosynthetic mutants (unpublished observations) which are very unstable with respect to both their photosynthetic phenotype and their plasmid profile. It is essential to point out that the observed plasmid instability may merely reflect a more general genomic instability, including chromosomal instability, which we are not in a position to measure directly. These observations point to the possible existence of an endogenous genetic element in *R. sphaeroides* 2.4.1 that is capable of affecting both plasmid stability and the photosynthetic phenotype. Thie endogenous genetic element may be a recombinational system that is sensitive to DNA damage or a stretch of DNA that has properties similar to an insertion element. The high frequency at which photosynthetic mutants arise in strain 2.4.1 suggests that the mutational element has a preference for affecting photosynthetic genes, with one class of mutants, those lacking light harvesting proteins, appearing at an unusually high frequency.

The presence of a genetic element capable of high-frequency alteration of the photosynthetic phenotype would seem to be a perfect complement to the physiological capabilities of *R. sphaeroides* in ecological competition. In many environments *R. sphaeroides* needs to change its growth mode from photosynthetic to aerobic respiration, anaerobic fermentation, or anaerobic respiration. Particularly in dark anaerobic environments, photosynthetic incompetent mutants, lacking an intracytoplasmic membrane, would be at an advantage since they would not need to expend energy assembling the gratuitous membrane system. Alteration of the light-gathering characteristics of the photopigments could also serve as a competitive advantage. Indeed this laboratory has isolated a *R. sphaeroides* strain from nature that has a "mutant" spectrum identical to that of L19.

Lastly, the nature of the reiterated sequences present on both pRS2.4.1e and one of the 99-kb plasmids remains to be determined.

ACKNOWLEDGMENTS

We thank M. B. O'Connor and N. Howe for providing strains and information before publication and C. Muster, J. Shapiro, and M. Howe for helpful discussions.

This work was supported by Public Health Service grant GM 31667 from the National Institutes of Health (to S.K.).

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