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Coliphage P1-Mediated Transduction of Cloned DNA from *Escherichia coli* to *Myxococcus xanthus*: Use for Complementation and Recombinational Analyses

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We have found that coliphage P1 can be used to transduce cloned DNA from *Escherichia coli* to *Myxococcus xanthus*. Transduction occurred at a high efficiency, and no evidence for DNA restriction was observed. The analysis of the transductants showed that they fall into three general categories: (i) haploid cells which contain portions of the cloned DNA substituted for homologous chromosomal DNA; (ii) heterozygous merodiploids which contain the recombinant plasmid integrated into the chromosome at a region of homology; and (iii) homozygous merodiploids which contain two copies of a portion of the cloned DNA with the loss of the chromosomal copy of the genes. The merodiploids, once formed, are relatively stable. They were used to analyze two genes necessary for aggregation and thus fruiting body formation. P1 transduction also permits the reintroduction and substitution of mutated regions of cloned DNA into *M. xanthus* for the analysis of the role of the DNA in cellular physiology and development.

Myxococcus xanthus is a rod-shaped gram-negative bacterium, commonly found in soils; it grows on decaying organic matter or by preying upon other microorganisms (21, 44). Under conditions of starvation on a solid surface, a developmental program is initiated. Cells reverse their outward movement and glide inward toward aggregation centers, where they accumulate into mounds. Rod-shaped cells within the mounds convert to ovoid or round myxospores. Mounds of myxospores are called fruiting bodies.

We have been interested in the genetic analysis of aggregation and sporulation functions (30, 38, 44, 45). Many of the traditional genetic procedures for the analysis of *Escherichia coli* genes are not currently available for *M. xanthus*. For example, conjugation and transformation are not reliable techniques in the laboratory. This makes genetic mapping and complementation studies rather difficult to perform. In fact, the only genetic transfer technique available for *M. xanthus* is generalized transduction, mediated by the *M. xanthus* phages Mx4 (7) or Mx8 and Mx9 (27).

Recently, Kuner and Kaiser (24) have shown that Tn*5*, a transposon which imparts kanamycin resistance, can be introduced into *M. xanthus* by using a broad-host-range coliphage, P1 (2), as a vector. Kaiser and Dworkin (20) showed that although *M. xanthus* is only distantly related to *E. coli*, over 99% of the phage particles adsorb to *M. xanthus* in 15 min. Although the adsorption and injection of DNA occur with high efficiency, the phage DNA is not stably maintained in *M. xanthus*, and phage particles are not formed. The insertion of Tn*5* near or into particular genes of *M. xanthus* is an extremely useful technique for the isolation of mutants and for mapping them by generalized transduction, since kanamycin resistance is a good selectable marker. Furthermore, the localization of a Tn*5* insert closely linked to a gene of interest in *M. xanthus* provides a selectable marker for the cloning of these genes in a plasmid vector in *E. coli*. This technique has been used successfully by several laboratories. To analyze these cloned genes further, however, we needed a method to reintroduce these genes into *M. xanthus*. In this paper, we report that bacteriophage P1 can be used as an efficient vector for the reintroduction of cloned *M. xanthus* genes into *M. xanthus*. Methods were also developed both for the screening and direct selection of merodiploids needed for complementation analysis. These results now permit sophisticated genetic analyses of particular regions of the *M. xanthus* chromosome.

MATERIALS AND METHODS

Bacteria and bacteriophage strains. *M. xanthus* DZF1 (an FB strain derived from DK101 [11]) was

TABLE 1. Bacterial strains

Strain designation	Relevant phenotype or genotype[a]	Tn*5* insert	Comment[b]	Source/reference
M. xanthus				
DZF1	Kan^s Tet^s Fru$^+$	None	Wild-type strain FB	(30)
DZF1516	Kan^s Tet^s Fru(Ts)	None	*aggR1*	(30)
DZF3080	Kan^r Tet^s Fru$^+$	Ω3080	Ω3080 is closely linked to *aggR1*	(38)
DZF3339	Kan^r Tet^r Fru$^+$	Ω3080	Merodiploid	By transduction of DZF1 with P1 grown on *E. coli*(pKO2)
DZF3340	Kan^r Tet^s Fru$^-$	Ω179	Gene replacement; Ω179 is in *aggR* region but not *aggR1*	By transduction of DZF1 with P1 grown on *E. coli* DZE179(pKO23-38)
DZF3341	Kan^r Tet^r Fru$^+$	Ω179	Merodiploid	By transduction of DZF1 with P1 grown on *E. coli* DZE179
DZF3342	Kan^r Tet^s Fru$^-$	Ω179	Gene replacement	By transduction of DZF1 with P1 grown on *E. coli* DZE179
DZF3343	Kan^r Tet^r Fru$^+$	Ω179	Merodiploid	By transduction of DZF1 with P1 grown on *E. coli* DZE179
DZF3350	Kan^r Tet^r Fru$^-$	Ω179	Merodiploid	By transduction of DZF1 with P1 grown on DZE179
E. coli				
C600		None		(1)
HB101	*hsdR recA*	None		(4)
SK1592	*hsdR*	None		(4)
DZE168		Ω3080	Contains plasmid pKO2 in C600	By transduction using recombinant DNA from DZF3080 cloned into the cosmid vector pHC79 (13)
DZE179	*hsdR*	Ω179	Contains plasmid pKO23-38 in SK1592	By insertion of Tn*5* into the *aggR* region of pKO2

[a] Fru(Ts), Temperature sensitive for fruiting body formation; Fru$^-$, nonfruiting phenotype at 28 or 34°C; Kan^s or Kan^r, sensitivity or resistance to kanamycin; Tet^s or Tet^r, sensitivity or resistance of oxytetracycline; *hsdR*, host specificity for restriction; *recA*, recombination ability.

[b] *aggR1*, Aggregation ability (loci mapped in reference 38).

used as the parental wild-type fruiting-competent strain (Fru$^+$). The strains of *M. xanthus* and *E. coli* used in this study are described in Table 1. For transduction experiments, P1 607H (39; obtained from John Roth, Department of Biology, University of Utah) was the principal bacteriophage used. For some experiments, P1 *virA* (17) or P1 Cm (33; obtained from John Clark, Department of Molecular Biology, University of California, Berkeley) was used. The recombinant *M. xanthus* plasmids used in this study are described in Table 2. More detailed information on these plasmids will be published elsewhere (K. O'Connor, B. Blackhart, J. Downard, and D. Zusman, manuscripts in preparation).

Media and growth conditions. *M. xanthus* cultures were grown on Casitone-yeast extract (CYE) broth (7) and aerated by shaking at 175 to 200 rpm at 30°C. Fruiting body formation was studied by spotting or plating cells on clone fruiting (CF) agar (11). *E. coli* was routinely grown in L-broth (29) or on L-plates which contained 1.5% agar (Difco Laboratories). The following special plates were used in the experiments: L-Ca, L-agar plus 5 mM $CaCl_2$; L-Tc, L-agar plus 20 μg of tetracycline (Sigma Chemical Co.) per ml; L-Kn, L-agar plus 50 μg of kanamycin sulfate (Sigma) per ml; L-Tc-Kn, L-agar plus 20 μg of tetracycline per ml plus 50 μg of kanamycin sulfate per ml; CYE-Tc, CYE agar plus 12.5 μg of oxytetracycline (Sigma) per ml; CYE-Kn, CYE agar plus 50 μg of kanamycin sulfate per ml; CF-Tc, CF agar plus 5 μg of oxytetracycline per ml; CF-Kn, CF agar plus 30 μg of kanamycin sulfate per ml; CF-Tc-Kn, CF agar plus 5 μg of oxytetracycline per ml plus 30 μg of kanamycin sulfate per ml. Since the plates containing oxytetracycline lost their potency rapidly, CF-Tc and CF-Tc-Kn plates were used within 2 days of being poured. CYE-Tc plates were stored in the dark at 4°C for not more than 2 weeks. It should be noted that oxytetracycline, rather than tetracycline, was used for selections involving *M. xanthus* because the former is less lethal to *M. xanthus* at intermediate drug concentrations.

Growth of bacteriophage P1 and transduction experiments. *E. coli* C600 or SK1592 harboring the plasmids was grown overnight in L-broth containing the appropriate drug: tetracycline (20 μg/ml), kanamycin (50 μg/ml), or ampicillin (25 μg/ml; Sigma). The cultures were diluted 1:20 into L-broth plus 5.0 mM $CaCl_2$ and incubated at 37°C. At 2×10^8 cells per ml, phage P1 was added to a multiplicity of infection (MOI) of 0.1. The cultures were incubated until lysis occurred. The lysates were shaken over chloroform and centrifuged at 12,000 × *g* for 10 min, and the supernatant was collected and stored at 4°C over chloroform. PFUs were determined by the method of Lennox (25) on L-Ca plates. We would like to note that liquid lysates were equivalent to plate lysates in their transducing titers. We did not find an increase in transducing titers when the lysates were prepared with cells pretreated

TABLE 2. Chimeric *M. xanthus* plasmids

Plasmid designation[a]	Vector	Size (kb)	Tn*5* insert	Drug resistance markers[b]	Source[c]
pKO2	pHC79	43	Ω3080	Tet^r Amp^r Kan^r	DNA from DZF3080 (Tn*5* linked to *aggR* genes) was cloned into pHC79 (13)
pKO23	pHC79	16.4	Fragment (left arm of Ω3080)	Tet^r	*Pst*I subclone of pKO3
pKO23-25	pHC79	22	Ω186 + fragment of Ω3080	Ter^r Kan^r	Tn*5* insertion (from λ::Tn*5*) into *aggR* region of pKO23
pKO23-38	pHC79	22	Ω179 + fragment of Ω3080	Tet^r Kan^r	Tn*5* insertion (from λ::Tn*5*) into *aggR* region of pKO23
pBB5	pHC79	30	Ω3150	Tet^r Kan^r Amp^r	DNA from DZF3150 (Tn*5* linked to frizzy genes) was cloned into pHC79
pJDK15-7	pBR322	18.3	Ω97	Amp^r Kan^r	Tn*5* insertion (from λ::Tn*5*) into plasmid containing protein S gene (the vector was pBR322 [5])
pJDK15-2C	pBR322	18.3	Ω142	Amp^r Kan^r	Tn*5* insertion (from λ::Tn*5*) into plasmid containing protein S gene
pJDK25-1a	pBR322	10.5	Ω137	Amp^r Kan^r	Tn*5* insertion (from λ::Tn*5*) into plasmid containing protein S gene
pJDK25-5c	pBR322	10.5	Ω139	Amp^r Kan^r	Tn*5* insertion (from λ::Tn*5*) into plasmid containing protein S gene

[a] The plasmids designated pKO were isolated by Kathleen O'Connor, pJDK was isolated by John Downard and Doris Kupfer, and pBB was isolated by Brian Blackhart.

[b] Tet^r, Resistance to tetracycline; Amp^r, resistance to ampicillin; Kan^r, resistance to kanamycin.

[c] *aggR* genes, Group of clustered genes involved in developmental aggregation (38); protein S genes, genes which code for the spore coat protein, protein S (18); frizzy genes, group of clustered genes involved in developmental aggregation (45).

with chloramphenicol to amplify plasmids (8).

The transductions with *E. coli* were performed as described by Lennox (25). The transductions involving *M. xanthus* were performed as follows. *M. xanthus* was grown in CYE broth to a concentration of 2×10^8 to 8×10^8 cells per ml. The cells were harvested by centrifugation at 7,000 × *g* for 5 min at 4°C and then suspended in 10 mM Tris-hydrochloride (pH 7.4) containing 10 mM $MgSO_4$ and 2.5 mM $CaCl_2$ at a density of 4×10^8 cells per ml. The adsorption of phage particles was performed by mixing 0.2 ml of cells with 1 to 500 μl of phage lysate (MOI < 1) and allowing the mixture to incubate at room temperature for 15 to 30 min without shaking. The cells were then plated on CF-drug plates (CF-Tc, CF-Kn, CF-Tc-Kn) or CYE-drug plates (CYE-Tc, CYE-Kn). The plates were incubated at 34°C for 5 to 10 days.

Plasmid purification and analysis. The method of Birnboim and Doly (3) was routinely used for the purification of plasmid DNA, except that an extra precipitation step (with 70% ethanol) was added after the suspension of the isopropanol precipitate. *M. xanthus* chromosomal DNA was extracted as described by Yee and Inouye (42) with several minor modifications. DNA was restricted with restriction endonucleases purchased from Bethesda Research Laboratories. The restricted DNA was analyzed on 0.7% agarose gels, transferred to nitrocellulose filters

TABLE 3. P1-mediated transduction of cloned DNA from *E. coli* to *M. xanthus*

Recombinant plasmid[a]	Size (kb)	Vector	No. of PFU/ml	No. of Kan^r transductants per ml	No. of transductants per PFU
pKO2	43	pHC79	1×10^8	9×10^4	9.0×10^{-4}
pKO23-25	22	pHC79	3×10^8	9×10^2	3.0×10^{-6}
pKO23-28	22	pHC79	2×10^6	9×10^2	4.5×10^{-4}
pBB5	30	pHC79	3×10^8	2×10^3	6.7×10^{-6}
pJDK15-7	18.3	pBR322	1×10^8	5×10^2	5.0×10^{-6}
pJDK15-2c	18.3	pBR322	7×10^7	1×10^3	1.4×10^{-5}
pJDK25-1a	10.5	pBR322	2×10^8	1×10^2	5.0×10^{-7}
pJDK25-5c	10.5	pBR322	8×10^8	<10	$<1 \times 10^{-8}$
None	—[b]	—	8×10^8	<10	$<1 \times 10^{-8}$

[a] P1 lysates were prepared on plasmid-containing strains of *E. coli* C600 or SK1592. A description of the plasmids is contained in Table 2. The titers of all lysates were determined on *E. coli* C600, and the lysates were tested for transduction on *M. xanthus* DZF1.

[b] —, None.

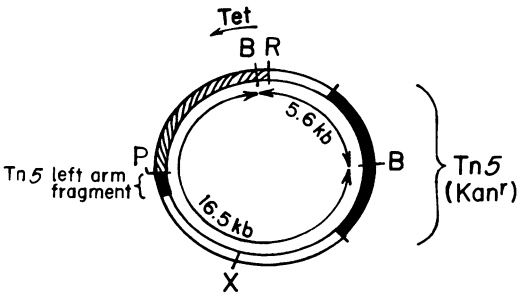

FIG. 1. Partial restriction map of plasmid pK023-38. Plasmid pK023-38 was constructed as part of a study of the *aggR* genes of *M. xanthus* (38) and will be described elsewhere (K. O'Connor and D. Zusman, manuscript in preparation). The plasmid contains a Tn*5* insert (shaded area) in the *aggR* region and therefore confers kanamycin resistance. It also contains a functional tetracycline resistance gene within vector pHC79 (hatched area). The restriction sites are as follows: B, *Bam*Hi; R, *Eco*RI; X, *Xho*I; P, *Pst*I.

(Sartorius), and hybridized to nick-translated probes by the procedures outlined by Davis et al. (9). For nick-translations (26), [^{32}P]dCTP (800 Ci/mmol; Amersham Corp.) was used.

For colony hybridization experiments, the method of Grunstein and Hogness (10) was used with several modifications. The colonies were transferred to Whatman 541 paper and treated sequentially for 2.5 min with 0.5 N NaOH (two times), 0.5 M Tris-hydrochloride (pH 7.4; two times), 2× SSC (9; 1× SSC, 0.15 M NaCl plus 0.015 M sodium citrate; two times), and 95% ethanol (two times). The filters were then air dried and hybridized to nick-translated probe as described by Davis et al. (9) for Southern blot transfers.

Screening, selection, and stability of *M. xanthus* merodiploids. *M. xanthus* merodiploids were screened by picking drug-resistant transductants onto CYE-drug plates. After 2 days at 34°C, they were replicated onto CYE-Tc plates. Screening by colony hybridization was performed as described above. Merodiploids could be selected by plating P1-infected cells onto CF-Tc or CF-Tc-Kn plates. Within 7 days, the colonies could be counted, and fruiting phenotypes could be scored after 10 days.

As a test of the stability of *M. xanthus* merodiploids, *M. xanthus* DZF3339 (Tet^r, Kan^r) was kept under constant selection on CYE-Tc-Kn plates and then grown for six generations in CYE-Tc-Kn broth at 30°C. The cells were collected by centrifugation (7,000 × *g* for 5 min at 4°C), washed with CYE broth, and suspended in CYE broth at about 10^7 cells per ml. The culture was incubated at 30°C; each day the culture was diluted with fresh medium so that the cell density never exceeded 4×10^8 cells per ml. At given intervals, small samples of the culture were diluted, plated on CYE plates, and incubated at 34°C. After 4 days, colonies were picked onto a grid. After 2 more days of growth, colonies were replicated onto CYE-Kn and CYE-Tc plates. All drug-sensitive colonies were checked a second time.

RESULTS

Use of coliphage P1 to transduce cloned DNA from *E. coli* to *M. xanthus*. Previously, we have reported a cluster of genes, which we have called the *aggR* loci (30, 38), involved in developmental aggregation in *M. xanthus*. Since a Tn*5* insert was located in one strain of *M. xanthus* immediately adjacent to this region, we were able to clone this DNA in *E. coli* by selecting for the kanamycin resistance element present in Tn*5* (manuscript in preparation). To locate the *aggR* genes within the chimeric plasmid, which was very large (43 kilobases [kb]), we needed a method to introduce the cloned DNA into *M. xanthus* so that the complementation or replacement of a mutant *aggR* allele could be obtained. Since transformation cannot be used for *M. xanthus*, we tried generalized transduction mediated by coliphage P1. The results of a typical experiment are given in Table 3. When the phage were grown on *E. coli* C600 containing the chimeric plasmid pK02, we obtained almost 10^5 kanamycin-resistant *M. xanthus* transductants per ml of lysate, i.e., 10^{-3} transductants per PFU. This frequency is better than that reported for plasmid transductions from one strain of *E. coli* to another (4×10^{-4} to 2×10^{-10} [16, 22, 31, 40, 41]) or from *Shigella* sp. to *E. coli* (31). Moreover, no precautions were taken in our experiments to minimize DNA restriction barriers presumed to be present between *E. coli* and *M. xanthus*. When the P1 lysate grown on *E. coli* DZE168, which contains plasmid pK02 (Table 1), was used on several restriction-negative (*hsdR*) *E. coli* strains, kanamycin-resistant transductants were obtained at the same frequency as that observed for *M. xanthus*. Thus, *M. xanthus* DZF1 apparently does not restrict *E. coli*-modified DNA.

Determination of capability of bacteriophage P1 to transduce other cloned DNA fragments from *E. coli* to *M. xanthus*. In our laboratory, we had available two other *M. xanthus* clones which could be tested. The first, pBB5, was a clone prepared by Brian Blackhart, using the same vector (pHC79), but containing the "frizzy" aggregation genes of *M. xanthus* (45; B. Blackhart and D. Zusman, manuscript in preparation). Table 3 shows that this clone can easily be transduced to *M. xanthus*, albeit at a lower frequency (2×10^3 transductants per ml). The second was a group of clones prepared by John Downard, which contain the two tandem copies of the protein S gene (18; J. Downard, D. Kupfer, and D. Zusman, manuscript in preparation). These clones were prepared with pBR322 as the vector (Table 2). The results in Table 3 show that the larger plasmids (18.3 kb) can readily be transduced into *M. xanthus*. Howev-

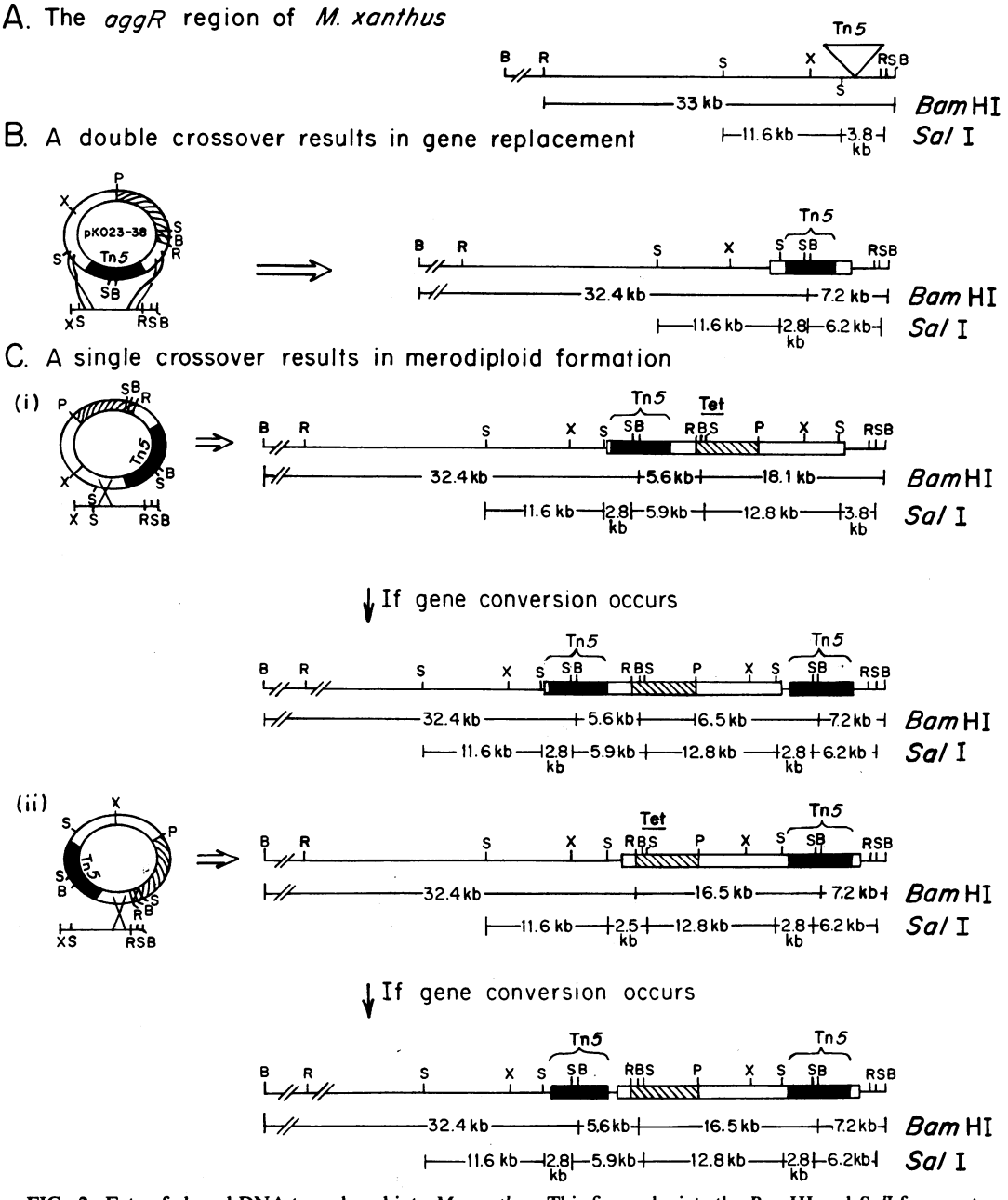

FIG. 2. Fate of cloned DNA transduced into *M. xanthus*. This figure depicts the *Bam*HI and *Sal*I fragments expected from gene replacement (B) and from merodiploid formation as the result of the integration of the cloned DNA into the *M. xanthus* chromosome [C (i) and (ii)]. The position of the Tn*5* of pK023-38 (Ω179) relative to the wild-type chromosome is shown in (A). Unshaded area, Cloned *M. xanthus* DNA; hatched area, vector DNA (pHC79); shaded area, Tn*5* DNA. The restriction sites are as follows: B, *Bam*HI; P, *Pst*I; R, *Eco*RI; S, *Sal*I; X, *Xho*I. All *Bam*HI and *Eco*RI sites are shown; only one of the *Pst*I and *Xho*I sites is shown, because they are convenient reference points. The *Sal*I sites in the left portion of the chromosomal DNA have been omitted since they are not relevant to the discussion.

TABLE 4. Preliminary complementation analysis of two fruiting loci[a]

Donor plasmid	Recipient *M. xanthus* strain	Phenotype	Rationalization	% Observed transductants	% Colonies which hybridized to pHC79 that were:	
					Tet^r	Tet^s
pKO23-38 (Kan^r Tet^r Fru^-)	DZF1 (Kan^s Tet^s Fru^+)	Kan^r Tet^s Fru^-	Gene replacement	8	—[b]	0
		Kan^r Tet^r Fru^+	Heterozygous merodiploids	67	100	—
		Kan^r Tet^r Fru^-	Homozygous merodiploids	25	100	—
pKO23-38 (Kan^r Tet^r Fru^-)	DZF1516 [Kan^s Tet^s Fru(Ts)]	Kan^r Tet^s Fru^-	Gene replacement	24	—	0
		Kan^r Tet^r Fru^+	Heterozygous merodiploids	50	100	—
		Kan^r Tet^r Fru^-	Homozygous merodiploids	26	100	—

[a] *M. xanthus* DZF1 or DZF1516 were transduced to kanamycin resistance (Kan^r) as described in the text. The transductants were tested for oxytetracycline resistance (Tet^r) and the ability to form fruiting bodies (Fru^+).
[b] —, None.

er, the smaller plasmids (subclones of 10.5 kb) could be transduced with difficulty or not at all. Thus, the relationship between the plasmid DNA and its ability to be transduced by bacteriophage P1 appears to be one of size: larger plasmids show better transducing efficiencies than smaller ones, provided that they are not larger than the P1 genome. This size relationship can also be seen in the transduction frequency of two subclones of pK02 (Table 3). These subclones, pK023–25 and pK023–38, are much smaller than the original clone (22 versus 43 kb) and have Tn*5* insertions at two different sites of the *aggR* DNA. Both of these subclones were transduced into *M. xanthus* at a much lower frequency per milliliter of lysate (about 100-fold) than was pK02. It should be noted that, in our hands, the number of transductants per milliliter of lysate was a fairly reproducible number from one lysate to another for the same plasmid; in contrast, the number of PFUs per milliliter often showed large variations. Given this variability, we feel that the number of transductants per milliliter of lysate is more significant than the ratio of transducing particles to PFUs. However, it was frequently observed that a high transducing titer was correlated with a low number of PFUs per milliliter. This may indicate that conditions which favor the formation of transducing particles reduce the formation of plaque-forming phage. We have not studied this aspect of lysate formation.

Fate of cloned DNA transduced into *M. xanthus*. In the experiments described in Table 3, bacteriophage P1 was used to transduce *M. xanthus* to kanamycin resistance. In these experiments, several possible fates of the donor DNA could lead to kanamycin resistance. First, the Tn*5* element could transpose from the plasmid DNA to a random location on the *M. xanthus* chromosome, with the remainder of the plasmid DNA lost or degraded. However, the known transposition rate from P1::Tn*5* to the *M. xanthus* chromosome is very low ($<10^{-8}$/PFU [24]) and could not account for the observed frequency of transduction. Second, the plasmid could be maintained and autonomously replicated. We attempted to detect new plasmids in the *M. xanthus* transductant strains by several methods (3, 9, 19) but were unsuccessful (plasmids derived from pBR322 generally have a limited host range [6]). Third, recombination events between homologous regions of the cloned DNA in the plasmid and the bacterial chromosome could result in the replacement of chromosomal DNA by cloned DNA. Fourth, the incoming plasmid DNA might circularize and then, by a single recombination event between an homologous region of the cloned *M. xanthus* DNA and the *M. xanthus* chromosome, cause the integration of the recombinant plasmid into the *M. xanthus* chromosome. This would result in the formation of a duplication of the cloned DNA sequence and the integration of vector DNA into the chromosome of the merodiploid.

To study the last two possibilities more closely, we decided to analyze the transduction of one plasmid in detail. Figure 1 shows a partial restriction map of recombinant plasmid pK023-38. This plasmid has a functional tetracycline resistance (Tet^r) element in the vector region and a kanamycin resistance (Kan^r) element in the Tn*5* insert, which lies within an aggregation gene (*aggR*), which is necessary for fruiting body formation. When DZF1 is transduced, one would predict (Fig. 2) that a double crossover which results in gene replacement would have a phenotype of Kan^r Tet^s Fru^- (kanamycin resistant, tetracycline sensitive, and fruiting body defective). In contrast, if a single crossover of a circularized plasmid results in plasmid integration and merodiploid formation, one would pre-

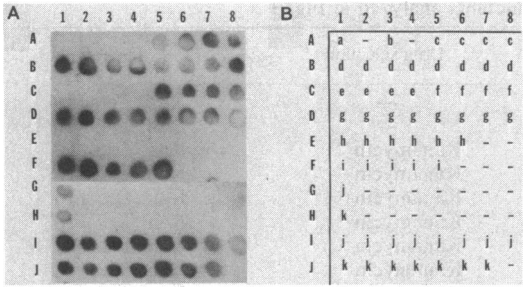

FIG. 3. Analysis of transductants for pHC79 DNA. Coliphage P1 was used to transduce *M. xanthus* DZF1 or DZF1516 to kanamycin or oxytetracycline resistance. The colonies were tested for the presence of vector (pHC79) DNA by colony hybridization to pHC79 DNA as described in the text. (A) Autoradiogram of the colonies; (B) key to the location of specific colonies. A dashed line indicates that no colony was present. The lower case letters refer to independent transductants tested from the same cross. A description of the transductants analyzed is given in Table 5.

dict a phenotype of Kan^{r}Tet^r Fru$^+$ (provided that the *fru* allele is recessive). Table 4 shows that both phenotypes were observed. About 92% of the kanamycin-resistant transductants are also resistant to oxytetracycline. All of these latter transductants contain vector DNA since they hybridize to nick-translated pHC79 DNA. Figure 3 shows sample colony hybridization analyses for some of these crosses, which are described in Table 5. The Kan^r Tet^s Fru$^-$ colonies (Fig. 3, e) did not hybridize with vector DNA and are consistent with being gene replacements. In contrast, the Kan^r Tet^r Fru$^+$ colonies (Fig. 3, d) all hybridized with the vector DNA and are consistent with being heterozygous merodiploids (the entire recombinant plasmid inserted into the bacterial chromosome) containing one wild-type copy and one mutant copy of the *aggR* allele. In addition, we observed a third group of transductants which had the phenotype Kan^r Tet^r Fru$^-$ (these usually comprised 10 to 30% of the Kan^r Tet^r transductants). These transductants hybridized with the vector DNA (Fig. 3, f) and are consistent with being homozygous merodiploids containing two mutant copies of the *aggR* allele. In these transductants, we think that the wild-type allele may be replaced by a mutant allele by gene conversion (12).

The confirmation of the genotypes of the three classes of transductants observed was obtained by Southern blot hybridization analysis of DNA extracted from representative transductants (Fig. 4). The parental strain, *M. xanthus* DZF1, has one *Bam*HI fragment which hybridized to the probe: nick-translated pK023 (this plasmid is identical to pK023-38 but lacks the Tn*5* insert Ω179; its size is 16.4 kb) (Fig. 4, lane 1). Figure 4, lane 2, shows the hybridization pattern of the probe to DZF3340. This transductant has the phenotype Kan^r Tet^s Fru$^-$ and was presumed to contain a gene replacement. Two *Bam*HI fragments from this strain hybridized to the probe: the larger fragment (32.4 kb) showed strong hybridization; the smaller fragment (7.2 kb) showed weak hybridization to the probe due to limited homology (it does not show up in Fig. 4 but did so in longer exposures of the blot). These size fragments agree with the predicted values shown in Fig. 2 for gene replacement. Figure 4, lane 3, shows the hybridization pattern of the probe to DZF3341. This transductant has the phenotype Kan^r Tet^r Fru$^+$ and was hypothesized to be a heterozygous merodiploid. The three hybridization bands obtained, 32.4, 18.1, and 5.6 kb, agree with the predicted values shown in Fig. 2C (i). A similar experiment was performed with the restriction enzyme *Sal*I (Fig. 5). This was done to allow better characterization of strain DZF3350, which has the phenotype Kan^r Tet^r Fru$^-$. We wanted to distinguish between the possibility that DZF3350 was a merodiploid which had become homozygous for the *fru* allele and the possibility that it was a heterozygous merodiploid of the type shown in Fig. 2C (ii). The predicted hybridization pattern for a *Sal*I digest in which gene replacement has occurred is three bands (11.6, 6.2, and 2.8 kb; Fig. 2B), and the predicted pattern for a heterozygous merodiploid (Fig. 2 C[ii]) is five bands (12.8, 11.6, 5.9, 3.8, and 2.8 kb). The pattern obtained for strain DZF3350 (Fig. 5, lane 3) showed five bands (12.8, 11.6, 6.2, 5.9, and 2.8 kb). This pattern is consistent with the predicted pattern for a homozygous merodiploid which has undergone gene conversion (Fig. 2). This pattern is not consistent with the incorporation of a multimeric plasmid (this possibility presupposes an additional 2.5-kb fragment, which was not observed).

Use of merodiploid formation for complementation analysis in *M. xanthus*. Since the transduction of cloned DNA into *M. xanthus* frequently results in the insertion of the chimeric plasmids into the homologous region of the *M. xanthus* chromosome, many transductants are heterozygous merodiploids in which the chromosomal copy and the plasmid copy of the genes of interest are tandemly arranged (Fig. 2). Distinguishing merodiploid transductants from haploid (gene replacement) transductants can be achieved by Southern blot analysis (Fig. 4) or, more easily, by colony hybridization to the vector DNA (Fig. 3). However, since merodiploids appear to express the tetracycline resistance element present on the vector (Tables 4 and

TABLE 5. *M. xanthus* transductants analyzed in Fig. 3

Code[a]	Donor	Recipient	Drug selection	Phenotype
a	—[b]	DZF1	—	Kan^s Tet^s Fru^+
b	—	DZF1516	—	Kan^s Tet^s Fru(Ts)
c	pKO2	DZF1	Kanamycin	Kan^r Tet^r Fru^+
d	pKO23-38	DZF1	Kanamycin	Kan^r Tet^r Fru^+
e	pKO23-38	DZF1	Kanamycin	Kan^r Tet^s Fru^-
f	pKO23-38	DZF1	Kanamycin	Kan^r Tet^r Fru^-
g	pKO23-38	DZF1516	Kanamycin	Kan^r Tet^r Fru^+
h	pKO23-38	DZF1516	Kanamycin	Kan^r Tet^s Fru^-
i	pKO23-38	DZF1516	Kanamycin	Kan^r Tet^r Fru^-
j	pKO2	DZF1	Oxytetracycline	Kan^s Tet^r Fru^+
k	pKO2	DZF1	Oxytetracycline	Kan^r Tet^r Fru^+

[a] The code letters refer to the key used in Fig. 3B.
[b] —, None.

6), we tried direct selection for merodiploids on plates containing oxytetracycline (Table 6 and Fig. 3). One hundred percent of those colonies which grew on oxytetracycline contained the vector DNA since they also hybridized with pHC79 DNA (Fig. 3, j and k). However, it should be noted that many of these transductants did not contain the kanamycin resistance marker (Table 6). The number of kanamycin-sensitive transductants was relatively high, usually about 30% (the data in Table 6 were somewhat atypical, showing 62% of the transductants as kanamycin sensitive). We think that the kanamycin-sensitive transductants were the result of gene conversion, in which the Tn*5*-containing portion of plasmid DNA is replaced by chromosomal DNA lacking Tn*5*, since gene conversion clearly takes place in the loss of the Fru^+ alleles described above. Direct selection for merodiploids can be achieved in these crosses by selecting for both oxytetracycline and kanamycin resistance. However, further analysis of the phenotype and genotype of the transductants is necessary to establish that gene conversion has not occurred.

If merodiploids are to be useful for complementation studies, they must be stable. Table 7 summarizes an experiment designed to study the stability of one such strain, *M. xanthus* DZF3339 (Kan^r Tet^r), under nonselective conditions. A culture of DZF3339 was pregrown in CYE broth containing kanamycin and oxytetracycline to assure that most of the cells (>99.3%) at the start of the experiment had both markers. The culture was then washed and suspended in nonselective medium lacking the antibiotics and maintained (with occasional dilution) in exponential-phase growth for 6 days. At specific intervals, the cells were plated out and tested for oxytetracycline or kanamycin resistance. As expected, some loss of kanamycin resistance and oxytetracycline resistance was observed. However, the rate of loss was not high: 0.2% per generation for oxytetracycline resistance and 0.1% per generation for kanamycin resistance (both groups of strains showed the same growth rate as DZF3339). These numbers agree with those reported by Shimkets et al. (35). The appearance of kanamycin-sensitive but oxytetracycline-resistant clones presumably would be the result of gene conversion. However, the rate of formation of these colonies was lower than what could be accurately assessed from the number of colonies counted in this experiment. This experiment shows that the merodiploid strains, once formed, are relatively stable.

Table 4 shows a preliminary experiment in which two loci in the *aggR* region were analyzed by complementation after merodiploid formation. Plasmid pK023-38 was generated by the transposition of a Tn*5* element into the *M. xanthus* DNA insert of the subclone pK023 (Table 2). When this plasmid was transduced into *M. xanthus* DZF1, the oxytetracycline-sensitive colonies, which do not contain vector DNA, (Figure 3, e) were all Fru^-. Since these Tet^s transductants result from gene replacements, the Tn*5* insert in this plasmid, Ω179, must rest in a fruiting gene in *aggR*. The Tet^r transductants all contained vector DNA (Fig. 3, d and f) and were merodiploids. Most of these transductants had the Fru^+ phenotype. These merodiploids must be heterozygous, containing both chromosomal and plasmid copies of the gene: they must have the mutated copy of the gene because these transductants are Kan^r (kanamycin resistance is conferred by the Tn*5* insert in the fruiting gene); and they must have a functional copy of the gene since the phenotype is Fru^+. Since the phenotype of the heterozygous transuctants is Fru^+, we conclude that the mutation generated by Ω179 is recessive. In

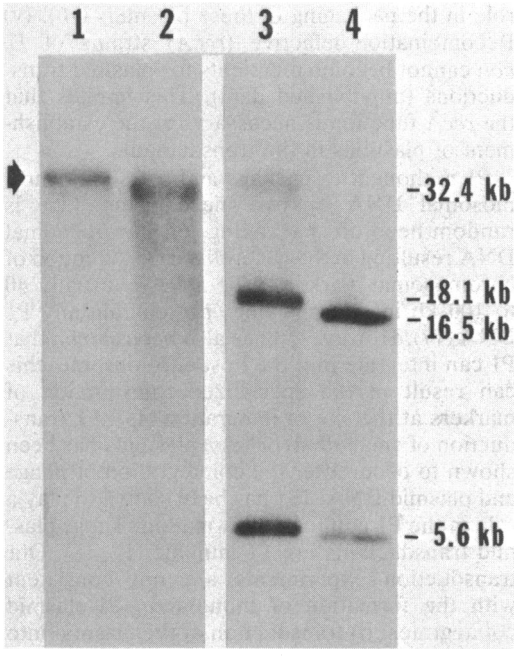

FIG. 4. Southern blot hybridization analysis of kanamycin-resistant transductants. *M. xanthus* DZF1 was transduced to kanamycin resistance by a P1 lysate grown on *E. coli* DZE179 (this strain carries plasmid pK023-38, depicted in Fig. 1). DZF3340 was a transductant which was Kan^r Tet^s Fru$^-$ (kanamycin resistant, tetracycline sensitive, and fruiting defective) and which did not hybridize to pHC79 DNA (Fig. 3). DZF3341 was a transductant which was Kan^r Tet^r Fru$^+$ and which did hybridize to pHC79 DNA. DNA was isolated from *M. xanthus* DZF1, DZF3340, and DZF3341, and from *E. coli* DZE179. The DNA was digested with the restriction endonuclease *Bam*HI, separated on a 0.7% agarose gel, and then subjected to Southern blot analysis (36). The blots were hybridized to a nick-translated probe of pK023 as described in the text. The lanes correspond to digested DNA from DZF1 (lane 1), DZF3340 (lane 2), DZF3341 (lane 3), and pK023-38 (lane 4). The arrow indicates the *Bam*HI fragment present in DZF1 (34.4 kb). The molecular sizes were calibrated from standards. Figure 2 shows the predicted molecular sizes for *Bam*HI fragments after gene replacement or merodiploid formation. Figure 1 shows the molecular sizes of the *Bam*HI fragments of pK023-38.

addition, 27% of the merodiploids had the Fru$^-$ phenotype. We think that these are homozygous merodiploids arising from gene conversion. Confirmation of the genotype of some of these transductants was obtained by Southern blot analysis similar to that shown in Fig. 5 (unpublished data).

Plasmid pK023-38 was also transduced into *M. xanthus* DZF1516 (Table 4). This strain is fruiting defective and was mapped previously in a locus called *aggR1* (38). In this cross, the Tet^s colonies were all Fru$^-$. These transductants result from gene replacements (they do not hybridize to vector DNA [Fig. 3, h] and contain Ω179 in a fruiting gene. The Tet^r transductants contain vector DNA (Fig. 3, g and i) and are merodiploids. Two phenotypes were observed among these transductants: Kan^r Tet^r Fru$^+$ and Kan^r Tet^r Fru$^-$. The Kan^r Tet^r Fru$^-$ transductants, 50% of the total, could only arise from the complementation of the two Fru$^-$ genes in heterozygous merodiploids. Therefore, the *aggR1* locus in DZF1516 must be recessive and lie in a different complementation group than the mutation caused by Ω179. At least some Kan^r Tet^r Fru$^-$ transductants (26% of the Kan^r transductants) were homozygous merodiploids, as indicated by Southern blot analysis. A more detailed study of the *aggR* region of *M. xanthus* is now in progress.

DISCUSSION

The results present in this paper show that coliphage P1 can be used to transduce cloned DNA from *M. xanthus* to *E. coli*. This is important because it provides the first reliable method for the introduction of recombinant DNA into *M. xanthus* for subsequent analysis. For example, one can construct recombinant DNA clones of *M. xanthus* genes and then transduce them into strains suitable for complementation analyses of heterozygous merodiploids. Additionally, cloned *M. xanthus* genes can be mutated (by Tn*5* transposition in *E. coli* or by in vitro modification of the DNA, for example) and then transduced into *M. xanthus* where the phenotype of the mutants (in haploid strains) can be ascertained. These are powerful techniques which, together with generalized transduction, should permit the rapid proliferation of genetic analyses in this interesting developmental organism.

In this paper, the transductions were usually carried out with a mutant of bacteriophage P1, P1 607H, prepared by Wall and Harriman (39), which reportedly shows increased (about 10-fold) levels of generalized transduction of chromosomal markers in *E. coli*. However, when lysates were prepared with P1 *virA* (17) or P1 Cm (33), essentially identical transducing titers were obtained for plasmid pK02. Thus, the ability of phage P1 to package plasmid DNA does not appear to be specific to one strain of phage P1.

How does phage P1 recognize and package plasmid DNA? The following observations made during our study may be relevant. (i) *E. coli* plasmid-transductants do not become P1 immune, nor are they inducible for P1. *E. coli* plasmid-transductants are indistinguishable from transformed plasmid-bearing *E. coli* in

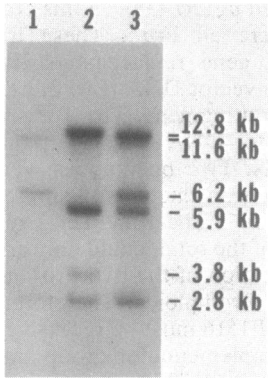

FIG. 5. Analysis of merodiploids by Southern blot hybridization of *Sal*I fragments to a pK023 DNA probe. *M. xanthus* DZF3340 (Kan^r Tet^s Fru$^-$), DZF3341 (Kan^r Tet^r Fru$^+$), and DZF3350 (Kan^r Tet^r Fru$^-$) were analyzed as described in the legend to Fig. 4 except that the DNA was digested with *Sal*I endonuclease rather than *Bam*HI. This digestion allowed us to characterize strain DZF3350, which appears to be a homozygous merodiploid containing two copies of the Tn*5* inserted gene of pK023-38. One copy is associated with the vector through merodiploid formation; the other has replaced the chromosomal copy of the gene (see the text). The lanes correspond to digested DNA from DZF3340 (lane 1), DZF3341 (lane 2), and DZF3350 (lane 3). The molecular sizes were calibrated from standards. Figure 2 shows the predicted molecular sizes for *Sal*I fragments after gene replacement or merodiploid formation.

making P1 lysates. The restriction digest patterns of plasmids isolated from *E. coli* transductants are identical to those from transformants. These observations are consistent with those of Kondo and Mitsuhashi (23) and Watanabe and Fukasawa (40). (ii) Plasmid transduction occurs efficiently at MOIs < 1. (iii) When P1 Cm was used as the transducing phage at a MOI of 0.2, <0.1% of *E. coli* plasmid-transduced cells were chloramphenicol resistant under conditions permitting P1 Cm lysogeny. The efficiency of plasmid transduction in *E. coli* was not reduced under conditions encouraging lytic infections of P1 Cm (41°C for our temperature-sensitive strain). In contrast, York and Stodolsky (43) have shown that, in the transduction of the 11-kb Tn*2901* (*argF*) as a cointegrate with P1 Cm0, 66% of Tn*2901* recipients are also chloramphenicol resistant. The transduction of Tn*2901* requires lysogeny or multiple infection. [Observations (i) to (iii) imply that the plasmids are not being transduced as stable cointegrates in the P1 genome.] (iv) The presence of Is*1* on a plasmid (e.g., in Tn*9*) does not affect the efficiency of transduction. (P1 has an IS*1* element [15].) This would seem to indicate that IS*1* does not play a role in the packaging of these plasmids (16). (v) Recombination-defective (*recA*) strains of *E. coli* cannot become recipients for plasmid transductions (unpublished data). This implies that the *recA* function is necessary for the establishment of plasmids in the transductants.

P1 is thought to package and transduce chromosomal DNA by two mechanisms. One is random-headfull packaging of chromosomal DNA resulting in the generalized transduction of chromosomal markers. The DNA is usually 40 to 100 kb in size and does not contain any P1 DNA (17). However, it has also been shown that P1 can integrate into the host chromosome; this can result in the specialized transduction of markers at the site of integration (43). P1 transduction of small (9 kb or less) plasmids has been shown to occur after the cointegration of phage and plasmid DNA. IS*1* has been shown to play a role in the P1 cointegrate formation. These plasmid transductants are P1 immune (15, 16). Our transduction experiments are not consistent with the formation of monomeric P1-plasmid cointegrates: (i) transduction of the plasmid into *E. coli* does not confer P1 immunity on the recipient cells; (ii) cointegrate formation should favor small plasmids, but our transductions strongly favor larger plasmids; and (iii) transduction experiments can be performed with MOIs several orders of magnitude less than one. (When a P1 cointegrate of R100 [>100 kb] was used for transduction, a MOI of 2 was needed to transduce it because no single particle contained sufficient DNA to allow recircularization by the recombination of homologous DNA [14].) Our data would be compatible with a modified P1-plasmid cointegrate model in which multimeric forms of the plasmid are involved in the cointegrates. (Plasmid preparations from *rec*$^+$ strains contain multimeric forms.) Packaging would presumably begin at the normal P1 "pack-site" and continue into plasmid DNA. The first particle packaged would be defective if it contained P1 DNA plus plasmid DNA greater than 9 to 10 kb since no terminal redundancy would be present for recircularization (16). However, DNA (headfulls) packaged subsequently from the P1-plasmid-multimer rolling circle would contain one copy plus some redundancy of any plasmid less than 90 to 95 kb in size. This redundancy would allow plasmid recircularization in the recipient cell (*recA* dependent) via homologous recombination.

An alternative model which does not involve cointegrate formation is also consistent with our data. This model would suggest that multimeric forms of the plasmid are packaged at random as is chromosomal DNA or that P1 may recognize a fortuitous pack-site in vector pBR322 and package headfulls form a multimer. We think that

TABLE 6. Expression of the tetracycline resistance gene from vector pHC79 in *M. xanthus* transductants[a]

Selective medium	No. of colonies scored	Kanamycin resistance (%)	Tetracycline resistance (%)	% Colonies which hybridized to pHC79 DNA that were:	
				Tet^r	Kan^r Tet^s
Kanamycin	250	100	41	100	0
Oxytetracycline	100	38	100	100	—[b]

[a] Coliphage P1 grown on *E. coli* DZE168 (C600 containing plasmid pKO2) was used to transduce *M. xanthus* DZF1 to kanamycin resistance or oxytetracycline resistance. The colonies were tested for oxytetracycline resistance (Tet^r) or kanamycin resistance (Kan^r) by picking onto CYE medium containing the tested drug. The colonies were tested for hybridization to a ^{32}P-labeled pHC79 DNA probe as described in the text.

[b] —, None.

fortuitous pack-sites are unlikely in the *M. xanthus* portion of the plasmids since transduction was successful with clones derived from at least three different regions of the *M. xanthus* genome. Although we have not worked with vectors unrelated to pBR322, the literature reports P1 transduction of plasmids whose derivations are independent of pBR322 (22, 31, 40). These researchers have found that plasmids are transduced by P1 with frequencies varying from 4×10^{-4} to 2×10^{-10}. Frequencies of transduction of plasmid R100 reported include 4×10^{-4} (40), 1×10^{-5} (41), and 1×10^{-6} (23) per adsorbed phage. Thus, the occurrence of fortuitous pack-sites is not limited to pBR322-derived plasmids. In general, plasmids are transduced at frequencies similar to those for chromosomal markers—10^{-6} to 10^{-7}/PFU (12). However, pK02 is transduced at a frequency of 10^{-3}/PFU. pBR322 may have a very favorable packlike site (heterologous pack-site) which causes it to be packaged by P1 at an elevated frequency.

The models which invoke multimeric forms of the plasmid are consistent with the increased efficiency of transduction which we observed for the larger plasmids. Dimers of a 43-kb plasmid would be sufficiently large to fill a P1 headfull (100 kb) with DNA. In contrast, at least an octomer or a nanomer of a 10-kb plasmid would be required to fill the same phage head. Phage P1 often packages small-headed particles containing 40 kb of DNA (17). These could be filled by a dimer of a 20-kb plasmid but would require a tetramer of a 10-kb plasmid. Since dimers of plasmids are much more common than larger multimers (32), the higher transduction frequencies observed for the larger plasmids may be related to the higher frequency of occurrence of these dimers compared with higher-order multimers. Small plasmids (<10 kb) could not be

TABLE 7. Stability of integrated chimeric plasmids in *M. xanthus*

Generations of outgrowth[a]	No. of colonies tested	Loss of drug resistance[b]							
		Tet^s		Kan^s		Tet^s + Kan^s		Kan^s + Tet^r	
		% of colonies tested	% Per generation	% of colonies tested	% Per generation	% of colonies tested	% Per generation	% of colonies tested	% Per generation
0	153	<0.7	—[c]	<0.7	—	<0.7	—	<0.7	—
1	217	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
8	297	2	0.25	1	0.13	0.7	0.09	0.3	0.04
13	708	2	0.15	1.4	0.11	0.9	0.07	0.3	0.02
24	294	5	0.21	2.4	0.10	1.4	0.06	1.0	0.04
30	733	4.2	0.14	2.2	0.07	1.9	0.06	0.3	0.01
36	327	5.8	0.16	1.8	0.05	1.5	0.04	0.3	0.01
Avg % per generation			0.2		0.09		0.06		0.02

[a] Generation times were monitored by absorbance. The average generation time under the experimental conditions used was about 4 h. DZF3339 had the same generation time in CYE and in CYE plus drugs.

[b] A culture of DZF3339, pregrown in CYE broth containing kanamycin and oxytetracycline, was suspended in CYE broth lacking these drugs. At specific intervals, samples were removed and tested for drug resistance as described in the text. Tet^s, Sensitive to oxytetracycline; Kan^s, sensitive to kanamycin; Tet^r, resistant to oxytetracycline.

[c] —, None.

successfully transduced by P1 in our experiments. Since some genetic analyses may require the use of smaller plasmids, we suggest that appropriate insertions, such as the 9.2-kb Tn*10*, in the vector region of small clones might be useful. This should permit the successful packaging of even the smallest subclones of *M. xanthus* DNA. A comparison of the work of Watanabe and co-workers (40, 41) with that of Iida et al. (16) also shows that size may play a role in the transduction of plasmids by P1. Small plasmids (6 to 7 kb) are transduced at frequencies of 8×10^{-6} to 3×10^{-7} per absorbed phage (16). However, R100 (>100 kb) is transduced at frequencies of 4×10^{-4} to 1×10^{-6} (23, 40, 41). R100 may be at the upper limit of the size of P1-transducible plasmids since the phage must contain more than the usual amount of DNA to give some redundancy for recircularization.

While this work was in progress, Gill and Kaiser developed a pBR322-derived vector (pREG411) which favors the formation of P1-plasmid cointegrates (R. Gill and D. Kaiser, personal communication). The vector contains a 6.7-kb *Eco*RI restriction fragment encoding P1-specific incompatibility (37). This vector has been successfully used by Shimkets et al. (35) for the transduction of cloned *M. xanthus* genes (*spoC* locus) from *E. coli* to *M. xanthus*. The frequencies of transduction observed by these workers were sometimes slightly higher than the ones reported in this paper. However, the results presented here show that it is not necessary to use a special vector since P1 packages pBR322-derived plasmids at sufficiently high frequency to be generally useful in the laboratory. Thus, we have been able to use the specialized vectors of Messing and his co-workers (28), which are also useful for Sanger sequencing work (34) for both genetic and sequence analysis of *M. xanthus* DNA (J. Downard and D. Zusman, unpublished data).

In this study, we were able to follow the fate of the cloned DNA, principally by analysis for vector DNA and by Southern blot hybridizations. We found three kinds of kanamycin-resistant transductants: (i) cells which had undergone gene replacement (these strains lacked vector DNA); (ii) cells which were heterozygous merodiploids (these strains contained vector DNA and both the chromosomal and plasmid copies of the cloned genes); and (iii) cells which were homozygous merodiploids (these strains contained vector DNA and two identical copies of the Tn*5* insertion). The Southern blots that we examined did not show any evidence of the transposition of the Tn*5* element to new sites on the chromosome, an event which had been shown to occur at low frequencies in *M. xanthus* (24).

The heterozygous merodiploids were found to be relatively stable. They are particularly useful for complementation analysis as illustrated in Table 4. The presence of a significant class of homozygous merodiploids was not expected. Like Shimkets et al. (35), we think that they originated during or immediately after the initial recombination step between circularized plasmid DNA and *M. xanthus* chromosomes by gene conversion. We attempted to measure the rate of gene conversion during exponential growth of *M. xanthus* by examining the rate of loss of kanamycin resistance in cells which maintain vector DNA (Tet^r) (Table 7). This rate was so low—much lower than the rate of loss of the vector DNA—that it could not be accurately measured in this experiment. The formation of homozygous merodiploids after the transduction of cloned DNA from *E. coli* to *M. xanthus* was also observed by Shimkets et al. (35) for the *spoC* loci. The presence of homozygous merodiploids adds complexity to complementation analysis which is not encountered in plasmid-associated merodiploids of *E. coli*. However, it is possile to perform clear-cut complementation studies with *M. xanthus* by carefully analyzing the transductants.

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