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# General Transduction in *Rhizobium meliloti*

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**General transduction by phage φM12 in *Rhizobium meliloti* SU47 and its derivatives is described. Cotransduction and selection for Tn*5* insertions which are closely linked to specific loci were demonstrated. A derivative of SU47 carrying the *recA*::Tn*5* allele of *R. meliloti* 102F34 could be transduced for plasmid R68.45 but not for chromosomally located alleles. Phage φM12 is morphologically similar to *Escherichia coli* phage T4, and restriction endonuclease analysis indicated that the phage DNA was ca. 160 kilobases in size.**

In recent years, a number of procedures for the genetic manipulation of fast-growing *Rhizobium* strains have been developed (4, 8, 10, 12, 18, 24, 27). Among the most intensively studied strains of *Rhizobium meliloti* are those derived from strain SU47, which was originally isolated by Vincent (32). In these strains, a circular linkage map of the chromosome has been constructed (24); mutants defective in nitrogen fixation ($Fix^-$) and nodulation of alfalfa ($Nod^-$) as well as various behavioral defects have been isolated (2, 15, 23); the organization and regulation of the *nif* operon(s) is being actively studied (27, 28, 30); and a DNA segment which contains nodulation genes has been cloned (21). However, none of three phages reported to perform general transduction in *R. meliloti* (phage 11, strain Rm41 [29]; phage DF2, strain GR4 [8]; phage L5, strain L5-30 [19]) forms plaques on SU47 derivatives. We therefore examined a number of new phage isolates for their ability to transduce these strains. This report describes the properties of one such phage and several examples illustrating its utility.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and media.** Table 1 lists bacterial strains and plasmids used.

LB and M9 media were as previously described (24). Soft-agar medium contained 8 g of nutrient broth (Difco Laboratories), 5 g of NaCl, 2.5 mM $MgSO_4$, 2.5 mM $CaCl_2$, and 6.5 g of agar (Difco) per liter of distilled water. Antibiotics were routinely used at the following concentrations (micrograms per milliliter): neomycin (Nm), 100 and 200; streptomycin (Sm), 250; rifampin (Rf), 50; novobiocin (Nv), 50; tetracycline (Tc), 10; spectinomycin (Sp), 100; and trimethoprim (Tp), 400.

**Sources of phage.** Phages φM1, φM5, and φM6 were from our laboratory collection. Phage φM7 was isolated directly from a precipitate (a gift from G. De Vos) obtained after polyethylene glycol treatment of soil from an alfalfa field. Phages φM9, φM10, φM11, φM12, and φM14 were isolated from commercial alfalfa inoculants manufactured in the United States (K. LeMieux, M.S. thesis, Northeastern University, Boston, Mass.). All phage isolates were plaque purified five times.

**Phage lysates.** In all experiments, the medium used for phage propagation was LB containing 2.5 mM $CaCl_2$ and 2.5 mM $MgSO_4$. Plate lysates were prepared by using the soft-agar overlay method (1). Confluent lysis occurred within 24 h. Phage was eluted from the plates by the addition of 4 ml of LB plus $Ca^{2+}$ plus $Mg^{2+}$ and incubating at 4°C. Phage φM12 liquid lysates were made by the addition of phage (either a plaque or ca. $5 \times 10^8$ PFU) to 5 ml of an actively growing culture (optical density at 675 nm, 0.4 to 0.5) and incubation at 30°C in a shaking incubator. Cell lysis normally occurred in 6 to 8 h. All lysates were sterilized with $CHCl_3$, centrifuged to remove cellular debris, and stored at 4°C in the absence of $CHCl_3$. Titers of $2 \times 10^{10}$ PFU/ml were routinely obtained, and during storage, the titers dropped at a rate of ca. 10-fold per month.

**Transduction.** Equal volumes of phage and bacteria (in LB plus $Ca^{2+}$ plus $Mg^{2+}$) were mixed to give a multiplicity of infection of ca. 0.5 phage per cell. The mixture was incubated at 30°C for 20 min. To select for the transduction of an antibiotic resistance marker, cells were centrifuged, suspended in LB, and plated directly on LB agar containing the antibiotic. For the transduction of prototrophic alleles, cells were centrifuged, washed twice with 0.85% NaCl, resuspended in 0.85% NaCl, and plated on minimal medium.

**DNA isolation and restriction endonuclease analysis.** Phage lysates prepared from 100-ml broth cultures of Rm5000 were treated with DNase (1 μg/ml), RNase (1 μg/ml), and $CHCl_3$ (3 ml) for 30 min at 30°C and then centrifuged at 16,000 × *g* for 10 min at 4°C. The supernatant fluid was removed and centrifuged in an SB-110 rotor for 1 h at 20,000 cpm (ca. 45,000 × *g*) in an IEC B-60 research ultracentrifuge. The resulting pellets were gently suspended in ca. 10 ml of MMC buffer (40 mM morpholinepropanesulfonic acid, 20 mM KOH, 4 mM $MgSO_4$, 1 mM $CaCl_2$ [pH 7.2]) and again centrifuged to remove bacterial debris. Phage was pelleted as described above, resuspended in MMC, and stored at 4°C. For DNA extraction, 20 mM EDTA was added, and the solution was extracted twice with phenol. The DNA was then dialyzed against 4,000 volumes of buffer containing 10 mM Tris-hydrochloride, 7 mM NaCl, and 1 mM EDTA (pH 7.8).

Restriction endonuclease digests were performed on ca. 4 μg of DNA in 200-μl reaction mixtures containing the buffers specified by the manufacturers of the restriction enzymes. Digests were analyzed by horizontal agarose (0.8%) gel electrophoresis in 40 mM Tris-acetate–2 mM EDTA (pH 8) buffer.

**Host range.** To determine whether φM12 could react with strains when plated at a high multiplicity of infection, ca. $10^8$ PFU was spotted onto double-layer indicator plates seeded with each strain. The ability of the phage to form plaques on these strains was examined by mixing cells and phage (ca. 200 PFU) in 0.2 ml of LB plus $Ca^{2+}$ plus $Mg^{2+}$; after 30 min,

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TABLE 1. Bacterial strains and plasmids

| Strain or plasmid | Relevant characteristics | Source or reference |
|---|---|---|
| *Rhizobium meliloti* | | |
| SU47 | Wild type | E. Johansen |
| EJ355 | SU47 *str-3 rif-101* | E. Johansen |
| Rm1023 | SU47 *str-21 met-1023*::Tn*5* | 23 |
| Rm1028 | SU47 *str-21 nod-1028* Nm$^r$ | 15 |
| Rm1145 | SU47 *str-21 nod-1145* Nm$^r$ | 15 |
| Rm1491 | SU47 *str-21 nifH*::Tn*5* | 28 |
| Rm2013 | SU47 *str-3 ilv-13* | 24 |
| Rm2111 | SU47 *str-3 cys-11* | 24 |
| Rm3330 | SU47 *str-3 spc-1 rif-1* | 24 |
| Rm3356 | Rm3330 *his-39 met-56* | 24 |
| Rm3357 | Rm3330 *his-39 leu-53 nov-57* | 24 |
| Rm3359 | Rm3330 *his-39 pyr-49 nov-59* | 24 |
| Rm3390 | Rm3330 *his-39 pan-44 nov-90* | 24 |
| Rm5000 | SU47 *rif-5* Nod$^+$ Fix$^+$ | E. Johansen |
| Rm5001 | Rm3330 Ω(*met-56*$^+$-*his-39*$^+$::Tn*5*)*5001* | This work |
| Rm5002 | Rm3359 *recA*::Tn*5* | This work |
| M5A | Pool of 5,000 Tn*5* insertions in EJ355 | This work |
| M5B | Pool of 1,500 Tn*5* insertions in EJ355 | This work |
| *Escherichia coli* | | |
| 1830 | *pro met nal* (pJB4J1) | 3 |
| HB101 | *rpsL pro leu thi recA hsdR hsdM* | G. Walker |
| J53 | *pro met nal* | E. Johansen |
| MM294A | *pro thi endA hsdR supE44* | G. Walker |
| *Plasmids* | | |
| pJB4JI | pPH1::Mu::Tn*5* | 3 |
| R68.45 | Ap$^r$ Nm$^r$ Tc$^r$ | 14 |
| pRMB1002 | pRK290 containing the *recA* gene of *R. meliloti* 102F34 with Tn*5* inserted in it | 5 |
| pRK2013 | ColE1 replicon containing transfer function of RK2 | 11 |
| R751 | Tm$^r$, P group plasmid | E. Johansen |

2.5 ml of soft-agar medium was added and poured on LB plus $Ca^{2+}$ plus $Mg^{2+}$ agar. Plates were examined after 48 h of incubation at 30°C. The following *R. meliloti* strains were tested and were obtained from the sources indicated in parentheses: L5-30 (M. Duncan); 102F51 (W. Brill); 102F34 (D. Helinski); Rm41 (A. Kondorosi); RCR2001, RCR2006, RCR2012 (Rothamsted Experimental Station, England); NA39, NA216, NA218-6, NA219, NA298, NA299-2, NA355, NA359, NA2290 (G. Bullard, Horticultural Research Station, Gosford, Australia); SU8, SU27, SU51/13L, SU66, SU101/W1, SU101/W2, SU126, SU134, R2, R3, R9, R10, R14 (M. Wilson, University of New South Wales, Australia); R11 (Z. Lorkiewicz).

**Electron microscopy.** Phage suspensions obtained after differential centrifugation (see above) were negatively stained with 2% uranyl acetate before viewing with a JEOL 100B electron microscope.

**Site-specific exchange of the Tn*5* insert in plasmid pRMB1002 with the *R. meliloti* chromosome.** Plasmid pRMB1002 was transferred from *Escherichia coli* HB101 to *R. meliloti* Rm3357 in a triparental mating with the mobilizing strain MM294A(pRK2013) and selection for rifampin- and neomycin-resistant colonies. The insert in pRMB1002 was homogenotized into the Rm3357 genome by mating with *E. coli* J53(R751), as described by Ruvkun and Ausubel (27). UV sensitivity was examined by streaking colonies across LB plates and irradiating with UV light; UV-insensitive cells grew after exposure to 40 J/m$^{-2}$, whereas sensitive cells did not grow after irradiation with 10 J/m$^{-2}$.

**Tn*5* mutagenesis.** Tn*5* insertions in EJ355 were obtained after mating with *E. coli* 1830 and selection on LB containing rifampin, streptomycin, and neomycin, as described by Beringer et al. (3). After two independent matings, 1,500 and 5,000 Nm$^r$ clones were pooled, and these pools were designated M5A and M5B, respectively.

## RESULTS

**Isolation of transducing phage.** Nine different phages which grew on SU47 were examined for transduction of Nm$^r$ from Rm1023 (*met-1023*::Tn*5*) to Rm5000. Seven of the phages gave no ($<10^{-8}$ per PFU) transductants. However, two phages, ϕM11 and ϕM12, transduced Nm$^r$ at $>10^{-7}$ per PFU. ϕM12, which produced a higher titer and more stable lysates, was examined further.

After overnight incubation at 32°C on soft-agar medium, ϕM12 formed clear plaques ca. 1.5 mm in diameter, which after a further 24 h became surrounded by a turbid ring and a halo. Attempts to isolate lysogenic bacteria from plaques were unsuccessful. Phage ϕM12 required $Ca^{2+}$ for infection, and no plaques were obtained on media without $Ca^{2+}$. In LB plus 2.5 mM $Ca^{2+}$ plus 2.5 mM $Mg^{2+}$ medium, >99% of the phage adsorbed in 20 min.

**Transduction.** Table 2 shows transduction by ϕM12 of markers from different regions of the SU47 genome. Transduction frequencies were of the order of $10^{-5}$ to $10^{-6}$ per PFU, and all transductants examined (ca. 130) were sensitive to ϕM12. Treatment of a ϕM12 lysate with DNase had no effect on the frequency of transduction. On LB plus $Ca^{2+}$ plus $Mg^{2+}$ agar, Nm$^r$ transductants appeared nibbled due to reinjection by active phage. To prevent killing of transductants on the plate, transduction mixtures were washed after adsorption (see above) and plated on media containing $Ca^{2+}$ at 0.25 mM or less.

Linkage of Nm$^r$ and Fix$^-$ from Rm1491 (Table 2) was expected, because the symbiotic mutation in Rm1491 results from the insertion of Tn*5* in the *nifH* gene (28). Strains Rm1028 and Rm1145 were obtained after Tn*5* mutagenesis (23), and although linkage of Nm$^r$ and Nod$^-$ was obtained from Rm1028, no Nm$^r$ transductants were obtained from Rm1145 as donor. This is presumably because as Buikema et

TABLE 2. Transduction of markers in derivatives of strain SU47[a]

| Donor | Recipient | Selected marker | No. of transductants per PFU |
|---|---|---|---|
| Rm1023 | Rm3330 | *trp-33*$^+$ | $2 \times 10^{-6}$ |
| Rm1023 | Rm5000 | Nm$^r$ | $2 \times 10^{-6}$ |
| Rm1023 | Rm3356 | *met-56*$^+$ | $1 \times 10^{-6}$ |
| M5B | Rm2013 | *ilv-13*$^+$ | $4 \times 10^{-6}$ |
| M5B | Rm2111 | *cys-11*$^+$ | $3 \times 10^{-6}$ |
| M5B | Rm3357 | *leu-53*$^+$ | $3 \times 10^{-6}$ |
| SU47(R68.45) | Rm3359 | *pyr-49*$^+$ | $3 \times 10^{-6}$ |
| Rm2013 | Rm3390 | *pan-44*$^+$ | $2 \times 10^{-6}$ |
| Rm1491[b] | Rm5000 | Nm$^r$ | $3 \times 10^{-6}$ |
| Rm1028[c] | Rm5000 | Nm$^r$ | $9 \times 10^{-6}$ |
| Rm1145 | Rm5000 | Nm$^r$ | $<10^{-8}$ |

[a] Transductions were performed as described in the text.
[b] Of 10 transductants examined, all were Fix$^-$.
[c] Of 10 transductants examined, all were Nod$^-$.

TABLE 3. Cotransduction of markers in *R. meliloti*

| Selected allele[a] | Coinherited marker | Cotransduction[b] (%) | Linkage by conjugation[c] (%) |
|---|---|---|---|
| *trp-33*$^+$ | *nov-59*$^+$ | 54 (396) | 92 |
| *met-56*$^+$ | *his-39*$^+$ | 5.4 (479) | 88 |
| *pan-44*$^+$ | *ilv-13* | 28 (315) | 83 |

[a] Donor strains were SU47(R68.45), Rm1023, and Rm2013, and the recipient strains were Rm3359, Rm3356, and Rm3390, respectively.
[b] The number of colonies examined is indicated in parentheses.
[c] Data from Meade and Signer (24).

al. (7) have shown, in strain Rm1145 phage Mu is inserted together with Tn*5*, whereas in strain Rm1028 no Mu sequences are present. Mu-Tn*5* inserts evidently cannot be transferred, as Meade et al. (23) have found in conjugation.

Cotransduction of the pairs of markers *trp-33-nov-59*, *met-56-his-39*, and *pan-44-ilv-13* is shown in Table 3. As the relative linkage values determined by conjugation and cotransduction should be similar, there is an apparent discrepancy in the values for *met-56-his-39*. However, Ames et al. (2) have reported a range of conjugal linkage values of 83, 53, and 37% for these same two markers. Moreover, differences in the relative linkage frequencies attained by conjugation and transduction could also be due to nonrandomness in phage packaging specificity.

In the experiments described below, a strain (designated Rm5001) was isolated in which Tn*5* was putatively linked to *met-56*$^+$ and *his-39*$^+$. Linkage of *met-56 his-39* and Nm$^r$ was studied further in this strain (Table 4). Although His$^+$ transductants could not be selected directly because the *his-39* mutation was leaky, the results were consistent only with the order *met-56*-Nm$^r$-*his-39*. Thus, ϕM12 transduction can be used to map bacterial loci. The presence or absence of Tn*5* between *met-56* and *his-39* in the donor strain had little effect on the *met-56-his-39* contransduction frequency (Tables 3 and 4). However, the length of Tn*5* (5.7 kilobases [kb] is likely to be small relative to the physical distance between the *met-56* and *his-39* mutations, which were only 5.4% cotransducible by a phage whose DNA was ca. 160 kb in size (see below).

Insertions of a drug resistance transposable element linked to a locus of interest serves to mark that locus genetically in subsequent transduction (13, 17, 20, 31). Transduction was used to show linkage of Tn*5* to *trp-33*, *met-56*, *ilv-13*, or *cys-11* (Table 5). For these experiments, pools of random Tn*5* insertions were prepared in EJ355 with the suicide plasmid

TABLE 4. Ordering of *met-56*, Nm$^r$, and *his-39* by ϕM12 transduction[a]

| Selected markers | No. of colonies scored | Class | % in class |
|---|---|---|---|
| *his*$^+$ Nm$^r$ | 229 | *met*$^+$ | 40 |
| | | *met*$^-$ | 60 |
| *met*$^+$ | 296 | Nm$^r$ *his*$^+$ | 5 |
| | | Nm$^r$ *his*$^-$ | 80 |
| | | Nm$^s$ *his*$^+$ | 0 |
| | | Nm$^s$ *his*$^-$ | 15 |
| Nm$^r$ | 352 | *met*$^+$ *his*$^+$ | 3 |
| | | *met*$^+$ *his*$^-$ | 73 |
| | | *met*$^-$ *his*$^+$ | 5 |
| | | *met*$^-$ *his*$^-$ | 19 |

[a] Donor strain: Rm5001 Met$^+$ Nm$^r$ His$^+$; recipient strain: Rm3356 *met-56* Nm$^s$ *his39*.

TABLE 5. Selection for insertion of Tn*5* near specific loci

| Recipient | Selected marker[a] | Nm$^r$ transductants[b] (% of total) | Cotransduction of selected marker with Tn*5*[c] (%) |
|---|---|---|---|
| Rm3359 | *trp-33*$^+$ | 0.8 | 70 |
| | | | 49 |
| | | | 20 |
| | | | 14 |
| Rm3356 | *met-56*$^+$ | 0.6 | 23 |
| | | | 76 |
| Rm2013 | *ilv-13*$^+$ | 0.7 | 51 |
| | | | 62 |
| Rm2111 | *cys-11*$^+$ | 0.7 | 55 |
| | | | 50 |

[a] For *trp-33*$^+$, the donor phage was grown on bacterial pool M5A; for other alleles, the donor phage was grown on bacterial pool M5B.
[b] The number of prototrophic transductants which were also Nm$^r$ was determined by replica plating onto LB containing 200 μg of neomycin per ml. For each marker, at least 1,000 prototrophic transductant colonies were screened.
[c] ϕM12 lysates prepared on purified Nm$^r$ prototrophic transductants were used to transduce the corresponding auxotrophs to Nm$^r$, and transductants were scored for prototrophy. Each entry represents a separate initial Nm$^r$ prototrophic transducant used to prepare a fresh lysate; 100 or more Nm$^r$ colonies were tested by replica plating in each cross.

pJB4J1 (3), and phage lysates were used to transduce each of the auxotrophic recipients to protrophy. In each case, 0.6 to 0.8% of the transductants were Nm$^r$. To confirm linkage of Tn*5* to each marker, a new lysate was grown on each of 10 of these transductants and then used to transduce Nm$^r$ back into the corresponding auxotroph. All 10 Tn*5* insertions were linked to the appropriate marker (Table 5, last column).

During these experiments, all Nm$^r$ transductants of Rm5000 were found to be Sm$^r$ as well. We subsequently confirmed that Tn*5* encodes a gene for Sm$^r$ expressed in *R. meliloti* but not in *E. coli* (9; also see reference 25).

**Construction of SU47 *recA*.** Better and Helinski (5) recently cloned a fragment of *R. meliloti* 102F34 DNA which complemented and had homology to the *E. coli recA* gene and inserted transposon Tn*5* into it. The putative *recA*::Tn*5* insert was then transferred to the chromosome by homogenotization, but although the resulting strain was UV$^s$, its proficiency in recombination was not examined (5). We have transferred that insert to the SU47 chromosome and then used ϕM12, first to transfer the insert to a *rec*$^+$ strain and then to show that a recipient with the insert cannot be transduced, as expected for a *recA* strain.

The *recA*::Tn*5* insert on plasmid pRMB1002 (Tc$^r$, Nm$^r$) was introduced into the chromosome of strain Rm3357 by homogenotization (see above). Of eight Nm$^r$ and Tp$^r$ transconjugants examined, all were Tc$^s$, indicating loss of plasmid pRMB1002, and also UV$^s$, indicating the presence of the *recA*::Tn*5* mutation replacing the wild-type gene. A ϕM12 lysate was then grown on one of these transconjugants and used to transduce Nm$^r$ to Rm3359. Whereas Rm3359 was UV$^r$, all of 20 transductants obtained at $2 \times 10^{-6}$ per PFU were UV$^s$. Thus, ϕM12 can be used to move the insert to new strains.

Next, one such transductant (Rm5002) was shown to be phenotypically *recA* as follows. A lysate of ϕM12 was grown on SU47(R68.45). The Tc$^r$ marker of R68.45 served as an internal control, as recombination should not be required for transduction of the entire plasmid. This lysate was then used to transduce strain Rm5002 and its *recA*$^+$ parent, Rm3359. In contrast to strain Rm3359, strain Rm5002 could not be transduced to *trp*$^+$ or *pyr*$^+$, although it could be transduced

TABLE 6. Effect of the *recA*::Tn*5* mutation on the transduction of chromosomal markers and plasmid R68.45 in *R. meliloti* Rm3359[a]

| Recipient | Relevant genotype | Transduction frequency | | |
|---|---|---|---|---|
| | | *trp*$^+$ | *pyr*$^+$ | Tc$^{rb}$ |
| Rm3359 | *trp-33 pyr-49* | $9 \times 10^{-6}$ | $5 \times 10^{-6}$ | $3 \times 10^{-7}$ |
| Rm5002 | *trp-33 pyr-49 recA*::Tn*5* | $<10^{-8}$ | $<10^{-8}$ | $1 \times 10^{-7}$ |

[a] Donor: SU47(R68.45) Trp$^+$ Pyr$^+$ Tc$^r$.
[b] Twenty Tc$^r$ transductants of both Rm3359 and Rm5002 were each crossed with *E. coli* MM294A. In all cases, the plasmids were conjugative, and the *E. coli* transconjugants were resistant to tetracycline, ampicillin, and neomycin.

to Tc$^r$ (Table 6). Thus, strain Rm5002 behaved as expected for a recombination-deficient *recA* strain.

**Properties of φM12.** Electron micrographs of negatively stained φM12 showed morphology resembling that of *E. coli* phage T4 (Fig. 1). The phage has an icosahedral head (diameter, 83 nm ± 5), a rigid and contractile tail (length, 100 nm ± 10; width, 18 nm) constructed of a hollow rod surrounded by ca. 23 to 25 annuli that end a short distance from the head, and a bar or collar at the base of the head. Thin fibers may extend from the neck-collar region. The baseplate has projections that might be short flexible fibers and also has very thin, long, tail fibers (50 to 60 nm). On particles with contracted tails, the regular pattern of annuli was confused, the diameter of the tail was increased, and the baseplate projections were larger.

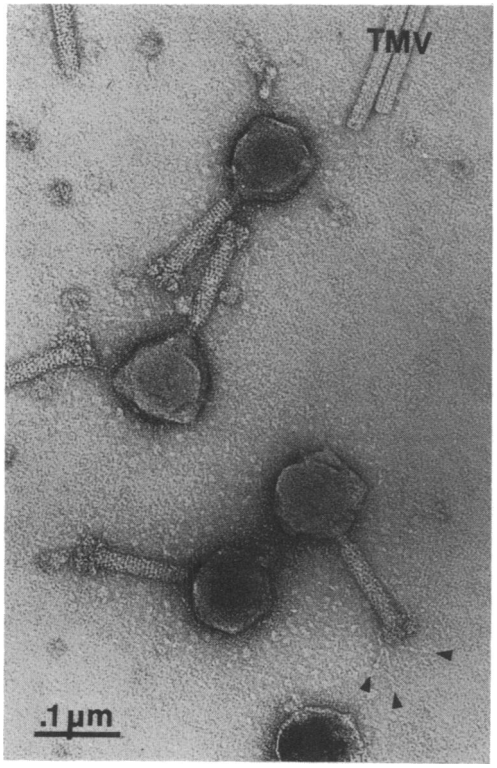


FIG. 1. Electron micrograph showing negatively stained φM12 phages. Note the tail fibers (arrowheads) and tobacco mosaic virus (TMV), which has a diameter of 1.8 nm and was used as a standard for size determinations.

DNA extracted from purified phage particles was examined for cleavage by a number of restriction endonucleases. No restriction was observed with *Bam*HI, *Bgl*II, *Kpn*I, *Mst*II, *Pvu*I, *Pst*I, *Sal*I, *Sma*I, *Stu*I, *Xba*I, or *Xho*I. Restriction fragments from *Acc*I, *Cla*I, *Eco*RI, and *Hin*dIII were separated on an 0.8% agarose gel (Fig. 2). Fragment sizes were estimated by comparison with fragments from *Hin*dIII cleavage of λ DNA. Addition of the fragment sizes for *Acc*I, *Cla*I, and *Hin*dIII, taking into account the multiplet structure of certain fragments, gave total length estimates of 176, 156, and 158 kb, respectively. *Eco*RI produced too many low-molecular-weight fragments for a meaningful estimate.

The host range of φM12 with respect to 30 *R. meliloti* strains was examined. When plated at a high multiplicity of infection, φM12 caused various degrees of clearing of all strains, indicating at least some adsorption and infection. However, the phage failed to form plaques on 12 of the strains, and plaques which varied in morphology from clear to very turbid were observed on the following strains: L5-30, 102F51, RCR2001, RCR2006, RCR2012, NA39, NA216, NA218-6, NA219, NA298, NA299-2, NA2290, SU51/13L, SU66, SU134, R2, R9, and R11.

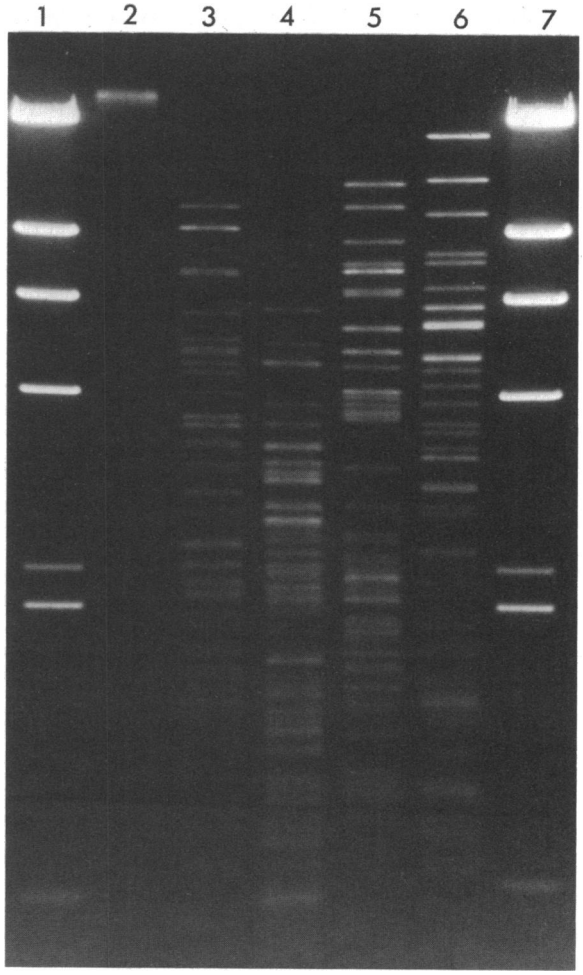


FIG. 2. Agarose gel electrophoresis of phage φM12 DNA digested with *Hin*dIII, *Eco*RI, *Cla*I, and *Acc*I (lanes 3, 4, 5, and 6, respectively). Lane 2 has unrestricted φM12 DNA, and lanes 1 and 7 have size standards from a *Hin*dIII digest of phage λDNA. Top to bottom: 23.0, 9.4, 6.5, 4.4, 2.3, 2.0, and 0.56 kb.

## DISCUSSION

Phage φM12 of *R. meliloti* is a large phage, morphologically similar to *E. coli* phage T4. Restriction analysis indicated a DNA length of ca. 160 kb, consistent with the size of the phage head. The failure of 11 of 15 restriction enzymes to cleave the DNA may mean the DNA is modified.

Phage φM12 carries out general transduction. Although the phage is virulent, reinfection of transductants can be prevented by omission of $Ca^{2+}$, which is required for adsorption. Markers located on the chromosome and the megaplasmid (21, 26) were transduced at $10^{-5}$ to $10^{-6}$ per PFU, and plasmid R68.45 (57 kb) (14, 16) could apparently be transduced in its entirety (Table 6). Marker pairs linked in conjugation can be cotransduced at frequencies comparable to those for the *Rhizobium leguminosarum* transducing phage RL38 (6). Phage φM12 has been used to order closely linked bacterial markers (Table 4), to identify Tn*5* inserts linked to specified markers (Table 5), and to construct and characterize an *recA* strain. Transduction by φM12 will considerably extend the range of genetic manipulations possible with *R. meliloti* SU47 derivatives.

In an accompanying paper, Martin and Long (22) describe phage N3, which also transduces in *R. meliloti* SU47 and its derivatives. We have found that rabbit antisera prepared against φM12 (inactivation constant, K = 184; see reference 1) also neutralized phage N3 (K = 138). This suggests that phages φM12 and N3 are related.

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