Specialized Transduction of a Cysteine Marker by *Rhizobium meliloti* Phage 16-3

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Specialized transduction by phage 16-3 of one of the *cys* markers of *Rhizobium meliloti* is described. The frequency of transduction was 10^{-6} to 10^{-7} per plaque-forming unit. About two-thirds of the transductants were heterogenotes, unstable for the *cys*⁺ marker, and yielded high frequency transducing lysates (10^{-2} per plaque-forming unit). In phage lysates, plaque-forming transducing particles were directly demonstrated while non-plaque-forming particles were only detected indirectly via complementation of defective lysogenic transductants. In the defective transductants, the two distal ends of the vegetative phage map were lacking. By plasmid transfer, the attachment site of phage 16-3 was located close to the *cys-46* and *met-5* markers. Transduction of the *met-5* marker could not be demonstrated.

INTRODUCTION

Fixation of atmospheric nitrogen in symbiotic association of legumes and *Rhizobium* species has recently attracted much interest. The genetics of *Rhizobium* is much less developed than the genetics of *Escherichia coli* or *Bacillus subtilis*, partly because of the limited possibilities for genetic transfer. Transformation (Balassa, 1963), generalized transduction (Kowalski, 1967) and, recently, chromosome mobilization mediated by plasmids (Beringer & Hopwood, 1976; Meade & Signer, 1977; Kondorosi *et al.*, 1977b) have been reported as possible methods for the genetic analysis of *Rhizobium*.

In this paper specialized transduction with phage 16-3 (Szendé & Ördögh, 1960; Orosz *et al.*, 1973) of *Rhizobium meliloti* 41 is described. Some of the results have been discussed previously (Sik & Orosz, 1971; Sváb *et al.*, 1973).

METHODS

Bacteria. The wild-type strain was *Rhizobium meliloti* 41. The auxotrophic mutants used were: A1 *his-1* (Orosz & Sik, 1970), F46 *cys-46* (Kondorosi *et al.*, 1974), AK178 *cys-46 met-5 str-3* and AK180 *cys-46 ade-3 str-3* (Kondorosi *et al.*, 1977b). Strain F46 is probably a sulphite reductase mutant, since it grows on minimal medium supplemented with cysteine, cystine or Na₂S but not with MgSO₄, Na₂SO₃ and homocysteine. Strains NG10 *cys-10*, K7 *arg-7* and K3 *trp-3* were obtained by treatment of *R. meliloti* 41 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Kondorosi *et al.*, 1973). Reversion rates of the mutants were 10^{-7} to 10^{-8} .

Bacteriophages. Wild-type phage 16-3 (Szendé & Ördögh, 1960), its thermoinducible (*ti3*, *ti4*, *ti5*) mutants in the immunity gene *c*, and thermosensitive (*ts5124*, *ts216*, *ts5116*, *ts518*, *ts5218*, *ts5222*, *ts2*, *ts5*) and lysis-deficient (*L51*) derivatives (Orosz *et al.*, 1973) were used.

Plasmid. Plasmid R68.45 was kindly provided by Professor B. W. Holloway.

Media. Yeast tryptone medium (YTB) contained (per litre): tryptone (Oxoid), 10 g; yeast extract (Oxoid), 1 g; NaCl, 5 g; CaCl₂, 1 mmol; MgCl₂, 1 mmol. Glucose ammonia minimal medium (BRP) contained (per litre) NaCl, 1 g; NH₄Cl, 3 g; glucose, 2 g; KH₂PO₄, 0.7 g; Tris, 3 g; HCl, 0.02 mol; biotin, 10 µg. The pH of the media was adjusted to 7.0 with NaOH (Orosz & Sik, 1970). For testing transductant colonies,

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BRP was solidified with 1.5 % (w/v) agar (Difco Noble). Glucose succinate ammonia minimal medium (GTPS), described by Kondorosi *et al.* (1977a), was modified by the addition of NH_4Cl (1 g l⁻¹).

Ultraviolet (u.v.) irradiation. Samples (2 ml) of bacteria or phage were suspended in saline plus 10 mM CaCl_2 in Petri dishes and irradiated with a 30 W Philips germicidal lamp at a distance of 90 cm.

Preparation of phage lysate. Lysogenic bacteria or bacteria infected by phage at a multiplicity of infection of 5 were grown to 2×10^9 to 4×10^9 bacteria ml⁻¹ at 28 °C in YTB broth supplemented with 10 mM CaCl_2 . The cultures were diluted twofold with the same medium and then either phage multiplication was induced or induction was omitted. For induction, *ti* lysogens were incubated for 40 min at 36 °C and *c*⁺ lysogens were u.v. irradiated for 10 s. After an additional 3 h growth period at 28 °C, Hyflo super cell (0.2 g ml⁻¹; Reanal Chemical Co., Budapest, Hungary) was added, and 15 min later the bacterial debris was sedimented by centrifugation. Chloroform-treated supernatant was used as the phage lysate. The lysate had a titre of about 10^{11} plaque-forming units (p.f.u.) ml⁻¹ when prepared from infected bacteria with or without induction, or from induced lysogenic bacteria. The titre of lysates from uninduced lysogenic bacteria was about 100-fold lower. Phage lysates were prepared from cultures set up from transductants and from turbid plaques as described for lysogenic bacteria.

Transduction technique. Recipient bacteria grown to 2×10^9 to 4×10^9 bacteria ml⁻¹ in YTB broth were diluted two- to threefold with YTB broth, and then the CaCl_2 concentration of the culture was raised to 10 mM. After 2 h further incubation, 0.9 ml of the culture was mixed with 0.1 ml phage lysate. After 30 min, 0.05 ml of the mixture was spread on to BRP agar plates and incubated for 3 to 5 d. All incubations were carried out at 28 °C.

Phage neutralization. Phage 16-3 rabbit antiserum (Orosz *et al.*, 1973) was used.

Bacterial mating. Donors and recipients grown overnight in YTB broth at 28 °C with aeration were mixed in a ratio of 1:1 and plated out on YTB agar. After incubation for 24 h at 28 °C, the bacteria were scraped off the surface of the plates, suspended in phosphate-buffer (0.02 M; pH 7.4) containing 0.9 % (w/v) NaCl and plated on selective media. Selective media were based on GTPS medium and supplemented, as required, with amino acids (50 µg ml⁻¹ adenine (25 µg ml⁻¹) and streptomycin (100 µg ml⁻¹). Recombinants appeared after 48 h incubation at 28 °C (Kondorosi *et al.*, 1977b).

RESULTS

Infection of the cysteine auxotroph strain F46 (*cys-46*) with lysates of phage 16-3 led to the appearance of prototrophic colonies at a frequency of 10^{-7} to 2×10^{-6} per p.f.u. Treatment of the lysates with serum prepared against phage 16-3 destroyed the effect but treatment with DNAase had no effect, suggesting this phenomenon to be a transduction. Transducing activity of the lysates was not dependent on the phage mutants used (*c*⁺, *ti*, *ts*) or on the nature of prophage induction (spontaneous lysis, u.v. or thermoinduction). Attempts to transduce several other markers, namely *cys-10*, *his-1*, *arg-7*, *trp-3*, *met-5* and *ade-3*, were unsuccessful. Conjugational mapping data (unpublished results) has indicated that *cys-10* and *cys-46* are about one-third of the *Rhizobium* chromosome apart.

Properties of Cys⁺ transductants

The Cys⁺ colonies (transductants) obtained were individually tested for the ability to give rise to lysates with high frequency transducing (HFT) activity. Of the lysates from 145 clones tested, 102 transduced the *cys-46* marker at high frequency (5×10^{-2} to 10^{-2} per p.f.u.) while 43 had low frequency transducing (LFT) activity (10^{-6} to 10^{-7} per p.f.u.). Transductants giving HFT lysates segregated Cys⁻ colonies during growth at a wide range of frequencies, indicative of heterogenotes. In contrast those which liberated LFT lysates were stable Cys⁺ and presumably haploid recombinants. Some properties of the transductants are summarized in Table 1.

Effect of phage multiplicity on transduction

A randomly chosen HFT lysate was used for transduction at different multiplicities (Fig. 1). The number of transductants increased linearly with the number of infecting phage, up to a multiplicity of infection (m.o.i.) of 5 to 10. The slope of the curve is consistent with the formation of transductants by one-hit, helper-independent events.

Table 1. *Properties of transductants*

Transduction was performed using phage lysates at an m.o.i. of 5 to 10. Phage lysates obtained from 145 transductant colonies were prepared either by thermoinduction (*ti4* and *ti5*) or without any induction (16-3). For the segregation test, transductant colonies were purified by re-streaking on BRP agar. One clone of each transductant was grown in YTB broth to 10^8 bacteria ml^{-1} and spread on to YTB agar to form 100 to 200 colonies per plate. After 40 h incubation, colonies were replica-plated on to BRP and YTB agar.

Phage lysate	No. of Cys ⁺ colonies tested	No. of colonies producing lysates of type:		No. of unstable clones per total examined	Percentage of Cys ⁻ progeny in unstable clones
		HFT	LFT		
16-3	34	20	14	5/5 0/2	1.5, 42, 34, 52, 65 —
<i>ti4</i>	34	28	6	3/3 0/2	18, 20, 31 —
<i>ti5</i>	77	54	23	5/5 0/4	22, 41, 46, 54, 81 —
Total	145	102	43	13/13 0/8

Fig. 1

Fig. 1. Effect of multiplicity of infection on transductant formation. Cultures of F46 grown overnight in YTB broth were diluted with YTB plus 10 mM-CaCl₂ to concentrations of 1.0×10^9 (○), 1.0×10^8 (●) and 1.0×10^7 (□) bacteria ml^{-1} , and used as recipients. Samples (0.9 ml) of each culture were mixed with 0.1 ml of HFT *ti3* lysates of different dilutions. After 30 min aeration, samples were spread on to BRP plates where transductants appeared after 3 to 4 d incubation at 28 °C.

Fig. 2

Fig. 2. Effect of u.v. light on viability and transducing activity of phage 16-3. HFT *ti3* lysate was irradiated by u.v. light for different periods. Transduction was carried out with these lysates as described in Methods. Ratios of transducing activity (●) and of plaque formation (○) of the irradiated sample to that of an unirradiated sample.

U.v. irradiation of an HFT lysate resulted in an exponential decrease in p.f.u. At low doses the transducing activity increased while at high doses it declined (Fig. 2). The increase has been attributed to an increase in the frequency of recombination between the host chromosome and the exogenote (Arber, 1958).

Nature of the transducing particles

To investigate whether the *cys*⁺ transducing particles were defective (*dc*) or plaque-forming (*pc*), six HFT lysates derived from independent transductants were plated out to form

Table 2. *Frequency of 16-3pc particles*

HFT lysates were allowed to form plaques on *r46* indicator bacteria on YTB agar by the agar-layer method. After 2 d incubation, the turbid centres of the plaques were spread with toothpicks on to BRP agar. *Cys*⁺ turbid centres formed colonies on BRP agar after incubation for 2 to 5 d at 28 °C.

HFT lysate	No. of <i>Cys</i> ⁺ turbid centres per total tested
HFT3	22/130
HFT109	0/147
HFT169	0/81
HFT174	0/45
HFT179	0/140
HFT181	0/43

Fig. 3. Map of the 16-3dc defective prophage. Solid lines represent regions present, dashed lines indicate regions deleted. (Map of 16-3 from Orosz *et al.*, 1973.)

plaques on the *cys* indicator strain *r46* on YTB agar. After 2 d, bacteria growing in the turbid centres of the plaques were spread with toothpicks on BRP agar for testing prototrophic growth (Table 2). Only one of the six HFT lysates gave *Cys*⁺ bacteria. This lysate, HFT3, evidently contained phage particles able both to produce plaques and to transduce the cysteine marker (16-3pc). Phage lysates were prepared from the turbid centres of 27 plaques of the HFT3 lysate, and transductions were performed at a m.o.i. of 0.1. Of the 27 lysates, 21 had HFT activity (5×10^{-1} to 1 per p.f.u.).

With the other five HFT lysates, no prototrophic bacteria were detected in the turbid centres of the plaques. Since the initial lysates had HFT activity, transducing and plaque-forming abilities were probably unlinked, so the phage population in these lysates consisted of separate defective transducing and plaque-forming particles. If this is correct, then defective lysogens should be found among transductants. To test this, transductions were carried out at a low m.o.i. (0.1 to 0.5) to avoid multiple infection and subsequent tandem lysogenization. Five *Cys*⁺ clones were isolated and studied further. None of the five clones liberated viable phages and all were sensitive to superinfection. We looked for defective phages in these transductants by complementation tests using a series of temperature-sensitive (*ts*) phage mutants with known map positions. Complementation analysis showed that the five transductant clones were defective lysogens. All of them contained genes from the middle part but not the two ends of the 16-3 vegetative chromosome (Table 3, Fig. 3). The five defective lysogens were superinfected with viable phages in an attempt to produce HFT lysates. Unexpectedly, the lysates had no HFT activity. Furthermore, the *Cys*⁺ phenotype of the transductants was stable, since no *Cys*⁻ segregants were found among 200 progeny tested from each of the five transductants.

Table 3. *Marker rescue in transductants unable to release viable phage particles upon induction*

Transductant clones were grown in YTB broth to 5×10^9 bacteria ml^{-1} and plated by the standard agar-layer method on YTB agar. After 1 h, lysates of different phages (0.02 ml ; 10^5 and 10^7 p.f.u. ml^{-1}) were dropped on to the plates. Lysis (complementation) was scored after 24 h at both 28 and 36°C .

Phage stock used for superinfection	Temp. ($^\circ\text{C}$)	Cys ⁺ clone and phage lysate used to produce it				
		D61 LFT <i>ts5116</i>	D45 LFT <i>ts5124</i>	D33 HFT <i>ti3</i>	D42 HFT <i>ti4</i>	D55 HFT <i>ti5</i>
Wild-type*	28	+	+	+	+	+
	36	+	+	+	+	+
<i>ts5124</i>	28	+	+	+	+	+
	36	+	—	—	—	—
<i>ts216</i>	28	+	+	+	+	+
	36	+	+	+	+	+
<i>ts5116</i>	28	+	+	+	+	+
	36	—	+	+	+	+
<i>ts518</i>	28	+	+	+	+	+
	36	+	+	+	+	+
<i>ts5218</i>	28	NT	NT	+	+	+
	36	NT	NT	+	+	+
<i>ts5222</i>	28	+	+	NT	NT	NT
	36	—	—	NT	NT	NT
<i>ts2</i>	28	+	+	+	+	+
	36	—	—	—	—	—
<i>ts5</i>	28	+	NT	+	+	+
	36	—	NT	—	—	—
<i>L51</i>	28	—	NT	NT	NT	NT

+, Lysis; —, no lysis; NT, not tested.

* Cys⁺ transductants are sensitive to superinfection which indicates the loss of *c*⁺ function.

Table 4. *Mapping of the att16-3 region on the Rhizobium chromosome*

Agar surface matings were performed as described in Methods. Counter selection for the donor was by streptomycin resistance. The frequency of transfer of the plasmid was about 3×10^{-1} . Recombinant colonies were purified by streaking for single colonies on selective media before testing for phage production as an unselected marker. Purified colonies were individually tested on indicator bacteria at 36°C where *c*⁺ phage produced turbid plaques and *ti3* phage produced clear plaques.

Donor	Recipient	Selected markers	Coinheritance of unselected (<i>ti3</i>) marker
<i>R. meliloti</i> 41(<i>ti3</i>) (R68.45)	AK178 (<i>c</i> ⁺ <i>cys-46 met-5 str-3</i>)	<i>met</i> ⁺ <i>cys</i> ⁺	182/182
		<i>met</i> ⁺	29/29
		<i>met</i> ⁺ <i>cys</i>	27/27
		<i>cys</i> ⁺	80/92
<i>R. meliloti</i> 41(<i>ti3</i>) (R68.45)	AK180 (<i>c</i> ⁺ <i>cys-46 ade-3 str-3</i>)	<i>ade</i> ⁺ <i>cys</i> ⁺	139/149
		<i>ade</i> ⁺	86/149
		<i>ade</i> ⁺ <i>cys</i>	0/16
		<i>cys</i> ⁺	121/138
		<i>cys</i> ⁺ <i>ade</i>	12/14

Localization of the att16-3 region on the Rhizobium chromosome by conjugation

The circular linkage map of the *R. meliloti* 41 chromosome was recently established (Kondorosi *et al.*, 1977*b*). *cys-46* and *ade-3* were cotransferred at a frequency of 0.57, and *cys-46* and *met-5* at a frequency of 0.76, and the order *ade-3 cys-46 met-5* was found. The *att16-3* region could therefore be located by determining the coinheritance of the *c* gene on the prophage with the *cys-46*, *ade-3* and *met-5* markers.

Plasmid R68.45 was introduced into *R. meliloti* 41 lysogenic for *ti3* and was used as a donor in matings with strains AK178 (*cys-46 met-5 str-3*) and AK180 (*cys-46 ade-3 str-3*) which were lysogenic for the wild-type phage. With selection for either *met*⁺ or *met*⁺*cys*⁺, all recombinants (100%) inherited the *ti3* allele. With selection for either *cys*⁺ or *ade*⁺*cys*⁺, however, only about 90% of the recombinants carried the *ti3* allele (Table 4).

DISCUSSION

Upon lysogenization, the genome of phage 16-3 integrates into the chromosome of *R. meliloti* near one of the cysteine genes, as demonstrated by the specific transduction of the *cys-46* marker and confirmed by conjugational data. That this transduction was specialized was established by the lack of transfer of six other auxotrophic markers. Furthermore, some of the Cys⁺ transductants (102 out of 145) were heterogenotes as described for other specialized transducing phages such as λ or ϕ 170 (Morse, Lederberg & Lederberg, 1956; Iijima, 1966). Heterogenotes yielded HFT lysates. The transducing activity of the 16-3 HFT lysates was 10⁻² per p.f.u. For comparison, the activity of λ or ϕ 80 HFT lysates was 10⁻¹ per p.f.u. (Morse *et al.*, 1956; Matsushiro, 1963) and that of P22, ϕ 170 or ϕ 81 HFT lysates was 10⁻³ to 10⁻⁴ per p.f.u. (Smith-Keary, 1966; Iijima, 1966; Takeda, Morishita & Yura, 1970).

These features suggest that the 16-3-*R. meliloti* system resembles systems capable only of specialized transduction, for example λ in *E. coli* (Morse *et al.*, 1956) and ϕ 80 in *E. coli* (Matsushiro, 1963), and clearly differs from other systems which are also capable of generalized transduction, for example P22 in *Salmonella typhimurium* (Smith-Keary, 1966), P1 in *E. coli*, P1 in *Shigella dysenteriae* (Luria, Adams & Ting, 1960) and 34 in *Proteus mirabilis* (Coetzee, 1974).

There were two types of transducing particles in the 16-3 phage lysates. The presence of plaque-forming *cys*⁺ (16-3*pc*) transducing particles was confirmed directly. Transducing particles of this type have been described previously, for example λ *pbio*, ϕ 80*pt* and ϕ 81*pg* (Wollman, 1963; Matsushiro, Sato & Kida, 1964; Takeda *et al.*, 1970). However, formation of defective *cys*⁺ transducing (16-3*dc*) particles was detected indirectly. Transductants raised from multiple infection liberated HFT lysates although they did not contain any *pc* particles. In these lysates, therefore, particles unable to form plaques had to be responsible for transduction. Furthermore, in some of the transductants from single infection, which did not yield any viable phages, phage genes mapping in the middle of the vegetative phage chromosome could be demonstrated by marker rescue (Table 3). The terminal ends and the *c* region coding for superinfection immunity were missing from the defective prophages. Although these transductants contained defective prophages, no HFT lysates could be obtained on superinfection. This is in contrast to all other known defective transducing particles, for example λ *dg*, P1*dl* and ϕ 80*dt*.

The properties of the 16-3*dc* particles can best be explained if *dc* particles can be integrated into, but not excised from the host chromosome. This possibility was supported by the observation that transductants from a single infection did not segregate Cys⁻ progeny, and no induction of HFT lysates was possible even on superinfection, although some of the 16-3 genes were still carried by the host chromosome. On the other hand, integration of the 16-3*dc* particle and the helper in tandem during multiple infection would make the production of HFT lysates possible, since integrated helper would exert not only a functional but also a structural effect on the excision and maturation of the *dc* particle.

An alternative explanation is that despite the predictions of the Campbell model, the Cys⁺ defective lysogens are a result of double crossover events at preferential sites between the transducing particle and the host chromosome. Lack of transduction of the *met-5* marker despite the fact that *att*16-3 is located closer to it than to *cys-46*, according to the cotransfer frequencies by the R68.45 plasmid, suggests that specific sequences might be located near to the *cys-46* region, which promotes recombination at that site.

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REFERENCES

- ARBER, W. (1958). Transduction des caractères *gal* par le bacteriophage lambda. *Archives des sciences, Geneva* **11**, 259–338.
- BALASSA, G. (1963). Genetic transformation of *Rhizobium*: a review of the work of R. Balassa. *Bacteriological Reviews* **27**, 228–241.
- BERINGER, J. E. & HOPWOOD, D. A. (1976). Chromosomal recombination and mapping in *Rhizobium leguminosarum*. *Nature, London* **264**, 291–293.
- COETZEE, J. N. (1974). High frequency transduction of kanamycin resistance in *Proteus mirabilis*. *Journal of General Microbiology* **84**, 285–296.
- IJIMA, T. (1966). Specialized transduction of the galactose markers by a temperate phage ϕ 170 in *Escherichia coli* k12. *Japanese Journal of Genetics* **41**, 121–130.
- KONDOROSI, A., BARABÁS, I., SVÁB, Z., OROSZ, L., SIK, T. & HOTCHKISS, R. D. (1973). Evidence for common genetic determinants of nitrogenase and nitrate reductase in *Rhizobium meliloti*. *Nature, New Biology* **246**, 153–154.
- KONDOROSI, A., OROSZ, L., SVÁB, Z. & SIK, T. (1974). Genetic studies on Rhizobiophage 16-3. II. Helper-induced transfection. *Molecular and General Genetics* **132**, 153–163.
- KONDOROSI, A., SVÁB, Z., KISS, G. B. & DIXON, R. A. (1977a). Ammonia assimilation and nitrogen fixation in *Rhizobium meliloti*. *Molecular and General Genetics* **151**, 221–226.
- KONDOROSI, A., KISS, G. B., FORRAI, T., VINCZE, E. & BÁNFALVI, Z. (1977b). Circular linkage map of *Rhizobium meliloti* chromosome. *Nature, London* **268**, 525–527.
- KOWALSKI, M. (1967). Transduction in *Rhizobium meliloti*. *Acta microbiologica polonica* **16**, 7–12.
- LURIA, S. E., ADAMS, J. N. & TING, R. C. (1960). Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. *Virology* **12**, 348–390.
- MATSUSHIRO, A. (1963). Specialized transduction of tryptophan markers in *Escherichia coli* k12 by bacteriophage ϕ 80. *Virology* **19**, 475–482.
- MATSUSHIRO, A., SATO, K. & KIDA, S. (1964). Characteristics of the transducing elements of bacteriophage ϕ 80. *Virology* **23**, 299–306.
- MEADE, H. N. & SIGNER, E. R. (1977). Genetic mapping of *Rhizobium meliloti*. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 2076–2078.
- MORSE, M. L., LEDERBERG, E. M. & LEDERBERG, J. (1956). Transduction in *Escherichia coli* k12. *Genetics* **41**, 142–156.
- OROSZ, L. & SIK, T. (1970). Genetic mapping of Rhizobiophage 16-3. *Acta microbiologica Academiae scientiarum hungaricae* **17**, 185–194.
- OROSZ, L., SVÁB, Z., KONDOROSI, A. & SIK, T. (1973). Genetic studies on Rhizobiophage 16-3. I. Genes and functions on the chromosome. *Molecular and General Genetics* **125**, 341–350.
- SIK, T. & OROSZ, L. (1971). Chemistry and genetics of *Rhizobium meliloti* phage 16-3. *Plant and Soil*, special volume, 57–62.
- SMITH-KEARY, P. F. (1966). Restricted transduction by bacteriophage P22 in *Salmonella typhimurium*. *Genetical Research* **8**, 73–82.
- SVÁB, Z., OROSZ, L., KONDOROSI, A. & SIK, T. (1973). Study of a specially transducing Rhizobium phage. *Acta microbiologica Academiae scientiarum hungaricae* **20**, 24.
- SZENDE, K. & ÖRDÖGH, F. (1960). Die Lysogenie von *Rhizobium meliloti*. *Naturwissenschaften* **47**, 404–405.
- TAKEDA, Y., MORISHITA, T. & YURA, T. (1970). Specialized transduction of the galactose genes of *Escherichia coli* by phase ϕ 81. *Virology* **41**, 312–355.
- WOLLMAN, E. L. (1963). Transduction spécifique du margueur biotine par le bacteriophage λ . *Comptes rendus hebdomadaire des séances de l'Académie des sciences* **257**, 4225–4226.