General Transduction in *Rhizobium meliloti* by a Thermosensitive Mutant of Bacteriophage DF2

JOSEP CASADESÚS AND JOSÉ OLIVARES*

Departamento de Microbiología, Estación Experimental del Zaidín, C.S.I.C., Granada, Spain

Received for publication 16 May 1979

A simplified version of bacteriophage DF2 transduction, using the thermosensitive mutant DF2*tsC*, is described. The transduction frequencies of *Rhizobium meliloti* markers by DF2*tsC* are of about 10^{-6} or higher. The development of transductants at 34°C does not require the addition of antiserum for phage neutralization.

Several recent developments on *Rhizobium* genetics, such as the construction of linkage maps (2, 5, 6), the detailed analysis of a specially transducing system (9), and the mutagenesis by transposable elements (1), have opened new expectations for the study of symbiotic nitrogen fixation in legumes.

The use of bacteriophage DF2 as a general transducer for the genetic analysis of *Rhizobium meliloti* has been previously described (J. Casadesús and J. Olivares, Mol. Gen. Genet., in press). The transduction technique by wild type phage is rather tedious, since it requires the addition of anti-DF2 antiserum to protect the growth of transductants. This can be simplified by using a thermosensitive derivative of phage DF2, called DF2*tsC*, which is capable of transduction with an equal or better efficiency than the wild type.

Bacterial strains used in this work were *R. meliloti* GR4 (Casadesús and Olivares, in press) and the derivatives listed in Table 1. Culture media were yeast-mannitol agar (4), yeast-sucrose agar, and yeast-sucrose broth, as described by Vincent (10), and the minimal medium of Hooykaas et al. (3). When required, minimal medium was supplemented with amino acids or base requirements to obtain a final concentration of 1 mM. In mutagenesis experiments, Tris-maleate buffer (pH 7.5) was used (A. Jiménez Sánchez, Ph.D. Thesis, University of Sevilla, Sevilla, Spain, 1974).

The DF2*tsC* mutant was obtained by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis. A log culture of strain GR4 was infected with wild type DF2 at a multiplicity of infection (MOI) of 5 and incubated for adsorption during 30 min. Then the culture was cooled, washed twice with Tris-maleate buffer, and resuspended at $80 \mu\text{g ml}^{-1}$ in Tris-maleate buffer plus *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Sigma).

Mutagen treatment was maintained for 60 min at 28°C with aeration. Afterwards, the culture was carefully washed with Tris-maleate buffer and incubated overnight in yeast-sucrose broth before the phage progeny were plated on yeast-sucrose agar.

The selection of phage mutants that were thermosensitive at 34°C was done by replica plating of lytic plaques. A mutant, DF2*tsC*, was tentatively considered for transduction purposes, since at the restrictive temperature it is able to adsorb but cannot lyse (mean values of three infection experiments: 2 lytic plaques at 34°C versus 3×10^9 at 28°C). The frequency of reversion to the wild type has been calculated as about 1.5×10^{-9} . The adsorption of phage DF2*tsC* on strain GR4 under normal and restrictive temperature conditions is of the same degree. In both cases, an extremely slow adsorption rate has been shown, as determined by counting the nonadsorbed particles present in a mixture of bacteria and phage after 20, 40, and 60 min of incubation. This behavior is similar to that shown by the wild type phage.

The transducing lysates have been obtained by the double-layer agar method (8) and titrated on yeast-sucrose agar. For transduction, bacterial cultures of about 10^8 cells ml^{-1} were mixed with transducing lysates to obtain the desired MOI and incubated for 40 min. Then the mixtures were centrifuged to discard the nonadsorbed particles and plated on selective media. Controls for phage sterility and for back-mutation of recipient strains were included. Lack of transduction when lysates were previously neutralized by serum has also been verified.

The highest transduction ratios were obtained at an MOI of 1 to 2. This is in agreement with the previous data on DF2 transduction (Casadesús and Olivares, in press). Therefore, the later transduction experiments were carried out

TABLE 1. *Derivative strains of R. meliloti GR4*

Strain	Genotype
GRC51 ^a	<i>phe-15 arg-17</i>
GRC52 ^b	<i>leu-5</i>
GRC54 ^a	<i>cys-4 thi-1</i>
GRC56 ^a	<i>ade-9 met-1 ura-2</i>
GRC38 ^a	<i>ala-1 ser-1 gly-10</i>

^a Strains described by Casadesús and Olivares (in press).

^b This paper.

at an MOI of about 1 (10^8 bacteria per 10^8 plaque-forming units).

DF2 and DF2tsC transducing abilities have shown to be of comparable efficiency according to the data obtained per plaque-forming unit for different genetic characters. The data on DF2tsC transduction only gave significant results when serum was added to the selective medium. The growth of transductants at the restrictive temperature did not require neutralization, since the phage progeny were unable to lyse.

The characteristics of DF2tsC transduction do not differ from those described for DF2. The effect of MOI on the transduction efficiency has been mentioned above. From 98 to 100% of transductants tested were stable for the transduced marker and did not segregate parental genotype clones. A high proportion of transductants (97 to 100%) were sensitive to the transducer phage. These properties indicate that DF2tsC acts as a general transducer.

The transductants showing phage resistance in phage sensitivity experiments were tested for lysogeny by ultraviolet irradiation and by mitomycin C induction. Ultraviolet treatments were done by irradiating 5 ml of a log culture with a 15 W Sylvania lamp for 2 s at a distance of about 15 cm. Mitomycin C (Sigma) treatments were done according to Otsuji et al. (7). Doses were 0.3, 0.6, and $1.2 \mu\text{g ml}^{-1}$. None of the transductants examined gave a lysate. Therefore, we have no hypothesis yet to explain the phage insensitivity, although the possibility of occasional

pseudolysogenic relations between *R. meliloti* and its phages could be considered (E. Corral, unpublished results). Nevertheless, most transductants show the typical behavior of arising from generalized transduction. The questions arising from the appearance of abnormal classes of transductants remain to be answered. In any case, this is an infrequent phenomenon and has little influence on transduction results.

We hope that transduction by DF2tsC will simplify the routine experiments of genetic analysis and will be appropriate for further fine-mapping studies of *R. meliloti*.

LITERATURE CITED

1. Beringer, J. E., J. L. Beynon, A. V. Buchanan-Wollaston, and A. W. B. Johnston. 1978. Transfer of the drug-resistance transposon Tn5 to *Rhizobium*. *Nature (London)* **276**:633-634.
2. Beringer, J. E., S. A. Hoggan, and A. W. B. Johnston. 1978. Linkage mapping in *Rhizobium leguminosarum* by means of R plasmid-mediated recombination. *J. Gen. Microbiol.* **104**:201-207.
3. Hooykaas, P. J. J., P. M. Klapwijk, M. P. Nuti, R. A. Schilperoort, and A. Rörsch. 1977. Transfer of the *Agrobacterium tumefaciens* T1 plasmid to avirulent *Agrobacteria* and to *Rhizobium explanta*. *J. Gen. Microbiol.* **98**:477-484.
4. Kleczkowska, J., P. S. Nutman, and F. A. Skinner. 1968. The identification and classification of *Rhizobium*, p. 51-65. In B. M. Gibbs and D. A. Shapton (ed.), *Identification methods for microbiologists*. Academic Press, London.
5. Kondorosi, A., G. B. Kiss, T. Forrai, E. Vincze, and Z. Banfalvi. 1977. Circular linkage map of the *Rhizobium meliloti* chromosome. *Nature (London)* **268**:525-527.
6. Meade, H. M., and E. R. Signer. 1977. Genetic mapping of *Rhizobium meliloti*. *Proc. Natl. Acad. Sci. U.S.A.* **74**:2076-2078.
7. Otsuji, M., M. Sekiguchi, T. Ijima, and Y. Takagi. 1959. Induction of phage formation in *Escherichia coli* K12 by mitomycin C. *Nature (London)* **184**:1079-1080.
8. Schwinghamer, E. A., and D. J. Reinhardt. 1963. Lysogeny in *Rhizobium leguminosarum* and *R. trifolii*. *Aust. J. Biol. Sci.* **16**:597-605.
9. Sváb, Z., A. Kondorosi, and L. Orosz. 1978. Specialized transduction of a cysteine marker by *Rhizobium meliloti* phage 16-3. *J. Gen. Microbiol.* **106**:321-327.
10. Vincent, J. M. 1970. A manual for the practical study of root-nodule bacteria, p. 43. IBP Handbook no. 15. Blackwell Scientific Publications, Oxford.