APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1989, p. 3229–3230
0099-2240/89/123229-02$02.00/0

Vol. 55, No. 12

Host Restriction and Transduction in *Rhizobium meliloti*

MYRON N. V. WILLIAMS, SHOSHANA KLEIN,† AND ETHAN R. SIGNER*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received 28 August 1989/Accepted 6 September 1989

A host restriction difference exists between *Rhizobium meliloti* Rm41 and SU47 exists as indicated by the reduce plating efficiency of transducing phage ϕM12h1. Restriction can be attenuated by incubating cells at 42°C for 3 h; this procedure overcomes a block to transduction from SU47 to Rm41.

The efficiency of plating of some bacteriophages is changed after growth on a new bacterial host. This host restriction phenomenon is nongenetic and, in *Escherichia coli* and *Salmonella typhimurium*, has been shown to result from cleavage of unmodified phage DNA by host restriction enzymes (1). Variation in physiological conditions may affect the degree of restriction. Here we describe an apparent host restriction system in the nitrogen-fixing alfalfa symbiont *Rhizobium meliloti* and conditions that inhibit restriction.

Rm41 and SU47 are different natural isolates of *R. meliloti* from Hungary and Australia, respectively. General transduction by phage ϕ11 in Rm41 has been reported, but this phage does not infect SU47 (13, 18). General transducing phage ϕM12 infects SU47 (5) but does not form plaques on Rm41, partly because it does not adsorb. A derivative of ϕM12 which plates on Rm41 was isolated following ethyl methanesulfonate mutagenesis (S. Klein, Ph.D. thesis, 1987). This phage, ϕM12h1, adsorbs nearly as well to Rm41 (in $CaCl_2$ at ≥10 mM) as to SU47 (data not shown), although on SU47 it forms smaller plaques and lysates with lower titers than ϕM12.

Phage ϕM12h1 grown on SU47-derived strains plates with reduced efficiency on Rm41 (5×10^{-2} to 5×10^{-3} plaques), while the same phage grown on Rm41 derivatives plates with slightly reduced efficiency on SU47 (1×10^{-1} to 5×10^{-2} plaques). Rm41 can be made sensitive to other SU47 phages such as ϕM9 by replacing its symbiotic megaplasmid pEXORm41 with pEXOSU47 (M. Williams, R. Hollingsworth, S. Klein, and E. R. Signer, manuscript in preparation). (Because the symbiotic megaplasmids of Rm41 and SU47 are lettered differently, we refer to homologous megaplasmids pRmeRm41c and pRmeSU47b as pEXORm41 and pEXOSU47, respectively.) Phage ϕM9 grown on SU47 also plates with an efficiency of 10^{-3} on Rm41 pEXOSU47 strains. This indicates that host restriction is not ϕM12h1 specific and that the genetic determinants of restriction are not located on pEXO.

Attenuation of restriction. The effect of the physiological state of cells on restriction was examined. Overnight 30°C Luria-Bertani (LB) cultures of Rm41-derived strains were treated in one of the following ways. They were grown at 30°C in LB for an additional 24 h (late stationary phase), diluted into LB to an A_{675} of 0.05 and shaken at 30°C for three generations (log phase), diluted into saline and shaken at 30°C for 10 h (starvation), grown in LB medium in sealed, nitrogen-flushed tubes at 30°C overnight (anaerobiosis), incubated at 4°C overnight, frozen at −70°C for 1 h and then thawed, incubated at 37°C for 10 h, or incubated at 42°C for 10 h (heat treatment). Only heat treatment, which does not alter the efficiency of plating on the homologous host, attenuated restriction, with 42°C being more efficient than 37°C (Table 1). Although *R. meliloti* heat shock proteins are induced within 5 to 10 min (A. Bent and E. Signer, manuscript in preparation), inactivation of restriction requires more than 1 h at 42°C (Table 2).

Transduction. A number of transposon-induced mutations located either on one of the two symbiotic megaplasmids or on the chromosome were introduced from SU47 to Rm41 by transduction. These included *nifH1491*::Tn*5* (8), *nodA9B8*::Tn*5* (10), *exoA61*::Tn*5-233* (3), *exoH154*::Tn*5* (12), *expA 8M*::Tn*5*, *expE2-17*::Tn*5-233* (7; J. Glazebrook, personal communication), *ndvB-TY1*::Tn*5-233* (4), *sxbB69*::Tn*5* (M. Williams, R. Hollingsworth, and E. Signer, manuscript in preparation), and *thi-501*::Tn*5-233* (6), with selection for resistance to 200 mg of neomycin per ml (Nm^r; Tn*5*) or to 65 μg of gentamicin per ml plus 5 μg of streptomycin per ml (Gm^r Sm^r; Tn*5-233*). Transduction from SU47 to Rm41 was very inefficient, averaging fewer than 10^{-8} transductants per PFU. However, when recipient Rm41 derivatives were first heat treated for 3 h, Nm^r or Gm^r Sm^r transductants were obtained at 10^{-6}/PFU. Since ϕM12h1 lysates seldom have titers higher than 10^8/ml, transduction is usually impossible without heat treatment. In each case, linkage frequencies between markers were comparable to those seen when ϕM12 was used in SU47, and transductants had the expected phenotypes.

TABLE 1. Effects of physiological conditions on plaque formation

Strain and cell condition	No. of plaques[a]	
	ϕM12h1 · SU47	ϕM12h1 · Rm41
Rm41		
Stationary phase	1	1,200
Log phase	5	
Starved	1	
Anaerobiosis	2	
Frozen	13	
4°C	1	
37°C	56	
42°C	1,050	1,200
SU47		
Stationary phase	1,200	65
42°C	1,200	1,500

[a] Absolute number of plaques formed from 100 μl of lysate in a representative experiment.

* Corresponding author.
† Present address: Department of Genetics, Hebrew University, Jerusalem.

TABLE 2. Time course of restriction attenuation

Strain and cell condition	No. of φM12h1 · SU47 plaques[a]
SU47	1,200
Rm41	
30°C	0
42°C, 15 min	1
42°C, 30 min	3
42°C, 60 min	80
42°C, 180 min	1,100

[a] Absolute number of plaques formed from 100 μl of lysate in a representative experiment.

Although heat treatment also increased the plating efficiency of φM12h1 · Rm41 on SU47 (Table 1), we have been unable to transduce any marker from Rm41 to SU47. We have not determined the reason for this, but heat treatment of SU47 decreased the efficiency of homologous transduction (from SU47) at least fourfold. Homologous transduction in Rm41 was also about 10-fold lower than in SU47 even without heat treatment, perhaps indicating that there are fewer transducing particles per phage grown in this strain.

Conclusions. These results indicate that *R. meliloti* Rm41 and SU47 have different host restriction systems. Restriction in Ti plasmid transfer to Rm2011, a derivative of *R. meliloti* SU47, has been suggested (17), and restriction of phage N3 has been reported in Rm1021 (SU47 *str-21* [13]), while restriction of transforming DNA has been observed in Rm41 (11). Nevertheless, we have shown that interstrain transduction can be accomplished by using a mutant phage and heat treatment. φM12h1 also infects other natural isolates of *R. meliloti* (102F34, 102F51, and L5-30), and this technique might be generally useful for interstrain genetic transfer.

In transformation, Selveraj and Iyer (17) proposed that a freeze-thaw cycle attenuated restriction in *R. meliloti* 102F54, but this does not appear to be the case here (Table 1). Transduction in *Streptomyces fradiae* is increased after growth at 39°C (relative to growth at 29°C), and the increase is greater in restricting than in nonrestricting strains (14). The capacity of *E. coli* K-12 to restrict phage λ is sensitive to UV irradiation (2) and heat treatment (16, 19), which inactivate the type I restriction enzyme *Eco*K (10). Our results are most easily explained by similar temperature sensitivity of restriction enzymes in *R. meliloti*.

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