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TRANSDUCTION IN *RHIZOBIUM MELILOTI*

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SUMMARY

Of 21 *Rhizobium meliloti* temperate phages, 12 transduced streptomycin resistance (*str-r*) with a frequency of 10^{-5} to 10^{-7}. Lysine dependence was transduced with frequency of 10^{-7}. Five transducing phages were used in experiments on the transfer of effectiveness from effective donor strain to 11 ineffective strains. However, plant tests did not reveal changes in recipient effectiveness.

INTRODUCTION

Genetic analysis of Rhizobium is reported using transformation [1] and recently conjugation [2]. Kowalski [4] found transducing phage L5 in *R. meliloti*. This paper reports the frequency of transduction in such phages and their ability to transfer streptomycin resistance (*str-r*) and lysine dependence (*lys*). Markers for effectiveness were not transferred.

MATERIALS AND METHODS

R. meliloti strain L5–30 which is effective, prototrophic and streptomycin-sensitive was the parent strain used, from which were obtained mutants resistant to streptomycin and lysine dependent and ineffective (L5–30 – 1 to 10). A lysine dependent mutant resistant to streptomycin was also ineffective (L5–30 lys).

Twenty-one temperate phages of *R. meliloti* described earlier[3] were used. Transducing lysates were obtained by multiplication on donors and UV irradiation of lysogenized donors.

Media and methods used in these studies were described earlier [4 5 6].

RESULTS AND DISCUSSION

From among 21 *R. meliloti* temperate phages tested for transduction of *str-r*, 12 transferred this marker with a frequency of 10^{-5} to 10^{-7} (Table 1). Both UV – induced and multiplied transducing lysates were active and this indicates a mechanism of general transduction. Ability of such phages to mediate general transduction was confirmed for phages L3, L5 and 13, which transduced *lys* marker with frequency 10^{-5} to 10^{-7}. The lysine dependent strain L5–30 *lys* was ineffective and linkage of these two markers was examined. It was found that both prototrophic

TABLE 1

Transduction of streptomycin resistance (*str-r*) by *Rhizobium meliloti* temperate phages

Phage	Transducing lysates obtained by			
	Multiplication		UV induction	
	Multiplicity of infection	Frequency of transduction	Multiplicity of infection	Frequency of transduction
L1	14.8	3.3×10^{-7}	109.0	2.7×10^{-5}
L3	6.4	3.5×10^{-7}	220.0	8.9×10^{-7}
L5	1.6	1.4×10^{-6}	20.0	1.0×10^{-6}
L21	0.25	1.9×10^{-5}	0.57	2.2×10^{-6}
L53	126.0	2.6×10^{-7}	116.0	2.6×10^{-7}
L54	42.5	2.8×10^{-6}	92.0	3.5×10^{-5}
L56	1.9	1.2×10^{-6}	88.0	5.4×10^{-6}
L57	74.0	4.0×10^{-7}	108.0	4.9×10^{-6}
L61	72.0	1.7×10^{-7}	131.0	4.5×10^{-6}
13	62.0	6.8×10^{-7}	366.0	2.0×10^{-5}
16	7.2	1.7×10^{-7}	5.9	5.5×10^{-6}
16a	25.0	1.6×10^{-6}	140.0	8.3×10^{-7}
L29	1.0	1.7×10^{-7}	84.5	0
L7	1.6	2.0×10^{-8}	0.9	0
L10	1.4	4.3×10^{-8}	10.7	0
L20	2.5	1.6×10^{-8}	10.0	0
L31	1.4	1.75×10^{-8}	116.0	0
L32	1.2	0	146.0	0
L55	9.6	0	10.0	0
L60	4.7	0	16.0	0
L62	1.1	0	15.0	0

Notes:

Recipient culture *R. meliloti* strain L5–30 *str-s* 1.8×10^8 cells/ml. Donor culture *R. meliloti* strain L5–30 *str-r*. The frequency of spontaneous mutation of the recipient to streptomycin resistance was 8×10^{-9}. 0 = no transduction.

TABLE 2

Results of plant tests with *Rhizobium meliloti* strain L5–30 wild type, its lysine-dependent mutant, and prototrophic revertants or transductants

Origin of *R. meliloti* strains	Number of clones		
	Tested	Effective	Ineffective
L5–30 wild type	5	5	0
L5–30 lys mutant	12	0	12
L5–30 lys* revertant	5	0	5
L5–30 lys* (L3)*	14	0	14
L5–30 lys* (L5)	6	0	6
L5–30 lys* (13)	5	0	5

* Symbol L5–30 *lys** (L3) denote transductants obtained with phage L3.

TABLE 3

Results of inoculation of lucerne plants with ineffective mutants of *Rhizobium meliloti* alone and treated with transducing phage L5

Recipient strain	Results of inoculation tests		
	Recipient*	Recipient + transducing phage L5**	
	Average number of nodules per plant	Average number of nodules per plant	Shape and colour of nodules
L5–30 *lys*	4	9	Small, white
L5–30–1	3	10	Small, white
L5–30–3	6	5	Small, white
L5–30–3	3	8	Small, white
L5–30–4	0	10	Long, white
L5–30–5	3	9	Small, white
L5–30–6	5	7	Long and small, white
L5–30–7	4	9	Long, white
L5–30–8	3	3	Small, white
L5–30–9	3	8	Very long and white reddish
L5–30–10	4	10	Very long and white reddish

Notes:

Recipient cultures containing about 2.0×10^8 cells/ml were mixed with transducing lysate of phage L5 having the titre 9.8×10^9 particles/ml in a ratio 1:1, and after 7 hr of incubation 0.2 ml of the mixture were inoculated into 5 tubes with 2 plants each.

* All plants inoculated with the mixture of the recipients with transducing phage L5 became nitrogen starved, indicating ineffectiveness of the inoculating clones.

** Transducing lysate of the phage L5 was obtained by multiplication on donor effective strain L5–30. Sterility of the lysate was confirmed by plating and inoculation test.

revertants and transductants were not effective (Table 2) indicating that these two markers are not closely linked.

For transduction of effectiveness, 11 ineffective strains were treated with transducing lysate of phage L5 and the mixture inoculated into sterile plants grown in tubes. Results of these tests (Table 3) showed differences in number and shape of nodules produced by the recipient strain in comparison to the control. However, all remained ineffective. Selection of possible effective transductants by plants may be difficult because of abundance of competitive ineffective recipients in the mixture [7].

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