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Transduction in the Archaebacterium *Methanobacterium thermoautotrophicum* Marburg

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The virulent bacteriophage ΨM1 of *Methanobacterium thermoautotrophicum* Marburg mediated transduction of a resistance marker and of three biosynthesis markers. Transductants were observed at frequencies of 6×10^{-4} to 5×10^{-6}/PFU.

Gene transfer systems reported for archaebacteria include mating (12, 14) and transformation with genomic (1, 4, 16), plasmid (2, 6), or bacteriophage DNA (3). Recently, gene transfer by a transductionlike process in *Methanococcus voltae* was reported (G. Bertani, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, I30, p. 222). Here we present evidence for transduction of the strictly anaerobic, thermophilic methanogenic bacterium *Methanobacterium thermoautotrophicum* Marburg mediated by bacteriophage ΨM1. To our knowledge, this is the first demonstration of transduction in an archaebacterium. ΨM1 is a virulent, oxygen-resistant phage specific for the Marburg strain of *M. thermoautotrophicum*. It is propagated with a latent period of 4 h and a burst size of six infective particles per cell (10). The linear, 30.4 kilobase phage DNA is circularly permuted and exhibits terminal redundancy of approximately 3 kilobases (7).

Wild type and mutants of *M. thermoautotrophicum* Marburg and bacteriophage ΨM1 are listed in Table 1. Bacterial mutants were obtained by established procedures (8, 9, 13), with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as a mutagenic agent. *M. thermoautotrophicum* was grown in minimal medium (10, 15), and bacteriophage propagation and titration were performed under anaerobic conditions, as described in detail elsewhere (7, 10). In all cases, crude bacteriophage lysates for transduction experiments originated from a single plaque of ΨM1 or ΨM2. Before infection, phages from a single plaque were suspended in 1 ml of minimal medium and passed through a membrane filter (Millipore Corp.) with a pore diameter of 0.45 μm to remove bacteria. The filtrate was added to 10 ml of an exponentially growing culture (7×10^6 CFU/ml), and incubation of the infected culture was continued under standard anaerobic growth conditions at 62°C until lysis occurred (usually within 1 to 2 days). Lysates were then treated with RNase A (5 mg/liter) and DNase I (25 mg/liter) for 30 min at 37°C outside the anaerobic chamber. In some experiments, the phage titer was increased by ultrafiltration through a PM30 membrane (Amicon Corp., Lexington, Mass.) to 3×10^8 PFU/ml.

For transduction, the appropriate strains (Table 1) were grown overnight to a density of approximately 10^8 cells per ml in 100-ml serum flasks containing 10 ml of minimal medium (15). If required, 10 mM L-leucine, 1 mM L-tryptophan, or 0.4 mM adenosine was included in the minimal medium. The serum flasks with the growing cultures were transferred to the anaerobic chamber, where all of the following steps of the transduction procedure were performed. Exponentially growing cells (5×10^6 or 5×10^7) were suspended in a final volume of 0.05 ml of minimal medium and gently mixed with 0.1 ml of an appropriately diluted (minimal medium) phage lysate. Dilutions of the lysate were chosen to obtain multiplicities of infection of 0.01, 0.05, and 0.1 for each experiment. The transduction mixtures were kept at room temperature for 10 min. Each mixture was then transferred into 2.3 ml of 0.6% soft agar at 60°C, poured onto agar plates, and incubated under selective conditions at 62°C as described elsewhere (10). Selection for pseudomonic acid resistance required 0.025 mg of pseudomonic acid A (Beecham Laboratories) per ml of medium. Resistance of *M. thermoautotrophicum* to this drug has been shown to reside in a mutationally altered, isoleucyl-tRNA synthetase (9). In the case of the doubly marked recipient strain MBT6, transductant colonies were scored for maintenance of the unselected pseudomonic acid resistance marker. Similarly, maintenance of auxotrophy was checked in pseudomonic acid-resistant transductants obtained from auxotrophic recipient strains (Table 2).

The transduction frequencies obtained with different ΨM1 lysates and for different markers are summarized in Table 2. They ranged from 5×10^{-6} to 6×10^{-4}/PFU. For each marker, the frequency of transduction varied by as much as a factor of 50, depending on the individual batch of phage lysate. The average transduction frequencies for particular markers (Table 2) were calculated from between two and six independent experiments, each one performed with a different lysate. By using the same lysate preparation kept at 20 to 25°C, transduction frequencies were reproducible, with an accuracy of ±25% within 7 days. Control plates with uninfected recipient cells were routinely maintained throughout the experiments, and revertants or spontaneously resistant colonies were never observed. The frequencies of revertants and of resistant mutants were below 10^{-8}. A phage lysate prepared on the Trp^- strain MBT6 did not raise the frequency of prototrophic colonies above 5×10^{-7} when MBT6 was infected (Table 2). This control demonstrated that phage infection itself has no mutagenic effect on the host bacterium. Transduction frequencies were dependent on the multiplicity of infection. A linear relationship between the number of transductants and the multiplicity of infection was observed at multiplicities of infection between 0.01 and 0.15 (data not shown). Experiments in which higher multiplicities of infection (0.5 to 5.0) were used yielded only a few or no transductants at all. Separation of the infected cells from nonadsorbed bacteriophages by a centrifugation step before the cells were plated did not improve the transduction frequencies at high multiplicities of infection. Under standard conditions, with multiplicities of infection between 0.01

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TABLE 1. *M. thermoautotrophicum* strains and phages

Strain or phage	Genotype or phenotype	Parent and/or reference
Strains		
MBT1	Wild type	5, 11
MBT2	*trp-30*	MBT1; 13
MBT6	*trp-30 ileS6*[a]	MBT2; 9, 13
MBT28	*leu-11*	MBT1; 8
MBT29	*leu-12*	MBT1; 8
MBT36	*ade-1*	MBT1; 8
Phages		
ΨM1	Wild type	10
ΨM2	Variant of ΨM1	7

[a] The *ileS6* (isoleucyl-tRNA synthetase) allele confers resistance to pseudomonic acid at an MIC of 2 μg/ml.

and 0.1, the transduction frequency was not significantly changed when the recipient cells were washed with minimal medium or when phage ΨM1 was replaced by phage variant ΨM2, which carries a 692-base-pair deletion in the genome (7).

Several characteristics of the observed gene transfer process indicated that this process was due to transduction. The gene transfer agent sedimented upon centrifugation of DNase I-treated phage lysates at 100,000 × *g* for 2 h. It was present in a bacteriophage preparation purified by CsCl equilibrium centrifugation (10), and the efficiency of the gene transfer process was not affected by DNase and RNase treatments of phage lysates. No transductants were observed when naked phage DNA or purified chromosomal DNA was substituted for phage lysate in the standard transduction protocol. Since the four chromosomal markers we have tested were successfully transduced at comparable efficiencies, it appears that bacteriophage ΨM1 carries out generalized transduction. This is in accordance with the fact that packaging of ΨM1 DNA proceeds from a *pac* site of a concatemeric precursor by a headful mechanism (7). The packaging machinery of phage ΨM1 exhibits relaxed specificity. ΨM1 efficiently encapsidates multimers of plasmid pME2001, which are present in strain MBT1 and its derivatives (10, 11), and nonspecific encapsidation of chromosomal DNA enables ΨM1 to act as a generalized transducing phage. The procedure reported here for generalized transduction may be useful for mutant analysis and strain construction in *M. thermoautotrophicum* Marburg.

TABLE 2. Transduction of biosynthesis markers and pseudomonic acid resistance with bacteriophage ΨM1 lysates[a]

Donor strain (phenotype)	Recipient strain (phenotype)	Trait selected for	Transductants/PFU	No. of expts
MBT1 (wt)	MBT6 (Trp^- Psa^r)	Trp^+	1.0×10^{-4}	6
MBT1 (wt)	MBT29 (Leu^-)	Leu^+	5.5×10^{-4}	3
MBT1 (wt)	MBT36 (Ade^-)	Ade^+	8.0×10^{-6}	2
MBT6 (Trp^- Psa^r)	MBT1 (wt)	Psa^r	1.0×10^{-5}	2
MBT6 (Trp^- Psa^r)	MBT28 (Leu^-)	Psa^r	6.0×10^{-4}	3
MBT6 (Trp^- Psa^r)	MBT29 (Leu^-)	Psa^r	4.0×10^{-4}	2
MBT6 (Trp^- Psa^r)	MBT36 (Ade^-)	Psa^r	5.0×10^{-6}	2
MBT6 (Trp^- Psa^r)	MBT6 (Trp^- Psa^r)	Trp^+	$<5.0 \times 10^{-6}$	2

[a] Recipient cells (5×10^6 or 5×10^7) were infected with phage ΨM1 crude lysates at a multiplicity of infection of 0.01 to 0.1. Psa^r, Resistance to pseudomonic acid A; wt, wild type.

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