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Transduction in Bacillus megaterium

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Summary: A bacteriophage, MP13, isolated from the soil on B. megaterium QM B1551 has been found to transduce several auxotrophic markers. Transduction required inactivation of the phage to approximately 0.01% survival with UV light and it was enhanced by the absence of salts that are probably necessary for phage readsorption.

INTRODUCTION

Transducing phages have been found for several species of Bacillus (1-8), and some of these have been used extensively to analyze B. subtilis sporogenesis. However, B. megaterium QM B1551 has the advantage of sporulating and germinating both completely and synchronously and has therefore been used to accumulate much biochemical data on differentiation (11-14). In addition, a variety of mutants (13-16) including germination mutants (17) are available. Unfortunately, genetic analysis of these mutants has not been possible since neither transformation nor transduction has been reported. Recently I have isolated and partially characterized 41 phages for B. megaterium (P.S. Vary, submitted). In this paper evidence is presented which suggests that one of these phages, MP13, is a generalized transducing phage. MP13 should therefore prove very useful in the genetic analysis of B. megaterium differentiation.

MATERIALS AND METHODS

Bacterial strains: B. megaterium QM B1551 was the host and indicator strain for MP13. Both wild type and auxotrophic mutants were obtained from Dr. J.C. Vary of the University of Illinois Medical Center and included JV45 (Pur-6), JV56 (Trp-1), JV61 (Trp-6 SmR), JV75 (Leu-1), JV78 (Leu-4 SmR) and JV94 (His-9) using the phenotypic designations as previously described (15).

Media: Bacteria and phage were grown in a rich medium, M, (18) unless otherwise noted. Phages were titered on M plates with 0.7% and 1.5% agar in top and bottom layers respectively. The minimal MC medium was previously described (13) and was modified to MCT medium by omitting the Ca^{++}, Mn^{++}, and Fe^{++} salts. Dilutions and UV exposures were made in phage buffer containing 8.7g K_2HPO_4, 2.72g KH_2PO_4, 0.246g $MgSO_4$ and 0.05g $CaCl_2$ per liter of distilled H_2O.

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Phage propagation: MP13 was propagated by adding 0.1 ml phage lysate to 25 ml broth containing 0.5 ml exponentially growing cells and incubated overnight at 30°C with shaking. Cells were removed by centrifugation at 2000 x g followed by filtration through a 0.45 µm membrane filter. A final concentration of 1% (w/v) peptone (Difco) was added to help stabilize the phage and lysates were stored at 4°C. Titers varied from 1 x 10^{10} to 5 X 10^{11} pfu/ml.*

Plate transductions: Approximately 5 x 10^8 pfu/ml of MP13 grown on wild type QM B1551 were spread on a minimal plate (MC or MCT) and irradiated with 43 ergs/cm^2/sec ultraviolet light at 254 nm. Auxotrophic recipients grown overnight on MC plus 20 µg/ml of their required supplement were picked to M broth plus 20 µg/ml of supplement and grown at 30°C with shaking to an optical density at 660 nm of 1.0 to 1.4. Then 0.3 ml of cells was spread over the irradiated phages and incubated at 30°C. Controls containing cells but no phage or phage but no cells were always run under identical experimental conditions. The first transductants appeared after 24 hours and were counted after 3 days incubation.

Tube transductions: Phages were diluted 1:10 in phage buffer to decrease the absorbance of UV light by the M medium and 0.5 ml of the dilution was irradiated in a 5 cm glass petri plate. The transduction mixture contained 0.1 ml of irradiated phage plus 0.9 ml cells at an optical density of 1.0-1.5. After standing 10 min to allow phage absorption, 0.1 ml of the mixtures was spread on MC or MCT plates and incubated as above.

DNase treatment: Transductions were also done following preincubation of the phages with 0.1 ml of 2 mg/ml pancreatic DNase (Sigma) in the presence of 5 mM $MgSO_4$ for 30 min at 37°C.

RESULTS

Phage isolation and characteristics - MP13 was isolated from soil along with 40 other phages and was partially characterized (P.S. Vary, submitted). Briefly, MP13 is a semi-temperate phage that can lyse several strains of B. megaterium including ATCC 10778, ATCC 11561A, ATCC 13368, KM and 899. It is a large phage with a polyhydral head, a contractile tail and several curly tail fibers. Recently it has been found to contain double stranded DNA (Halsey and Vary, unpublished results).

Effect of UV light and salts on transduction - A typical set of data is shown in Table 1 in which phages grown on wild type were used to transduce JV78 (Leu-4 SmR). First, as controls, no transductants were observed without phages or without inactivation of the phages with UV light (Table 1A, lines 1 to 3). However, when phages were exposed for 15 sec to UV light (approximately 0.01% survival of phages, data not shown), a significant number of transductants were

*Abbreviations; pfu: plaque forming units; cfu, colony forming units; SNB, supplemented nutrient broth (13).

Table 1

Transduction of leucine prototrophy

Donor	Phage before UV (pfu/plate)	JV-78 Recipient (cfu/plate)	UV (sec)	Prototrophs on medium: MC	MCT	Transductants/pfu
A	0	3.9×10^7	0	1	3	0
	0	3.9×10^7	15	0		0
wild type	8×10^8	3.9×10^7	0	0	0	0
	8×10^8	3.9×10^7	15	56		7.0×10^{-8}
	8×10^8	3.9×10^7	45	47		5.9×10^{-8}
	1.6×10^9	3.9×10^7	45	72		4.5×10^{-8}
	8×10^8	3.9×10^7	15		700	8.8×10^{-7}
	8×10^8	3.9×10^7	15		636	8.0×10^{-7}
B	pfu/tube	cfu/tube				
wild type	8×10^7	1.4×10^8	0		0	0
	8×10^7	1.4×10^8	15		30	3.8×10^{-7}
	8×10^7	1.4×10^8	30		15	1.9×10^{-7}
	8×10^7	1.4×10^8	45		14	1.8×10^{-7}
	8×10^7	1.4×10^8	60		0	0
C	0	3.9×10^7	15		4	0
JV-78	3×10^9	3.9×10^7	15		7	1.0×10^{-9}

A. Plate transductions are described in Methods. Phage were grown on wild type QM B1551 and JV78 was the recipient. B. Tube transductions were done as described in Methods using phages diluted 1:10 in phage buffer before UV exposure. C. A self cross in which donor and recipient was JV78.

found. The number of transductants was proportional to the number of phages added when adequate inactivation was obtained (see line 6). All control plates contained from 0-4 revertants and all lysates were free of cells. Finally, many more transductants were obtained when MCT medium, which lacked the heavy metals of MC was used. This suggests that phage readsorption to transductants was probably inhibited which further enhanced transductant survival.

As shown in Table 1B, when less than 15 sec of UV exposure was used, no transductants were observed. Exposure to UV light for greater than 15 sec decreased the number of transductants, presumably because of damage to the transducing DNA. As an added control (Table 1C), a self-cross was made in which MP13 grown on JV78 was used to transduce the same strain. There was no significant increase in transductants (control = 4, self cross = 7) in contrast to the 700 colonies observed when a wild type donor was used (Table 1A).

Table 2

Effect of DNase on Prototroph Appearance

Phage (pfu/plate	Cells (cfu/plate)	$MgSO_4$ (ml)	DNase (ml)	UV (sec)	Prototrophs
0	2.9×10^7	0.1	0	20	3
8×10^8	2.9×10^7	0.1	0	20	586
8×10^8	2.9×10^7	0.1	0.1	20	596
8×10^8	3.8×10^7	0.1	0	20	270
8×10^8	3.8×10^7	0.1	0.1	20	540

Phages grown on wild type were exposed to UV light on a plate, then 0.05 M $MgSO_4$ and DNase (2 mg/ml) were spread over the phages. Plates were incubated 30 min, 37°C and cells of strain JV-78 were spread on the plate. Prototrophs that were streptomycin resistant were counted after 3 days.

Table 3

Transduction of Several Auxotrophic markers

Recipients	Prototrophs (cfu/plate)	
	Control	Phage
JV45 (Pur-6)	5	29
JV61 (Trp-6 SmR)	12	120
JV75 (Leu-1)	20	360
JV78 (Leu-4 SmR)	3	642
JV94 (His-9 SmR)	10	492

Plate transductions were made as in Table 1 with either 0.1 ml H_2O as a control or with phages exposed 15 sec to UV light. All recipients were between 2.7 and 3.9×10^7 cfu/plate. Phage inactivation was not complete in all cases, e.g. JV45, since plaques were observed on the thin confluent background.

In these experiments, transductants were picked to MC plates or to rich SNB plates containing 100 µg/ml streptomycin. Greater than 99% of the colonies

tested remained prototrophic, and were still streptomycin resistant, therefore ruling out contamination by wild type cells.

Effect of DNase on transduction - Table 2 gives the results of experiments in which DNase was incubated with the irradiated phages in the presence of Mg^{++} before transduction. All reagents were free of cells. As can be seen, prototrophic colonies still appeared at the same frequency therefore ruling out the possibility that transformation occurred instead of transduction.

Demonstration of generalized transducing ability of MP13

A lysate of MP13 grown on wild type QM B1551 was used to transduce several recipients with different auxotrophic requirements and the results are shown in Table 3. It is apparent that several different markers can be transduced and that MP13 is therefore a generalized transducing phage.

Discussion

From the data presented it is evident that MP13 is a generalized transducing phage for B. megaterium. Several different genes can be transduced including Trp, His, Pur and Leu. Moreover, transductants are proportional to the number of phage present and are resistant to DNase. The maximum ratio of transductants/pfu is about 8×10^{-7} but this frequency should increase as optimal conditions are developed. For instance, antiserum to MP13 is being made that should further enhance the recovery of transductants. It is evident that a simple method has been found to transduce B. megaterium QM B1551. There are several auxotrophic mutants already available in this species and we are rapidly isolating more for genetic mapping. MP13 has an added advantage of being a large phage and therefore may carry a large amount of bacterial DNA. In addition to mapping, MP13 should be very useful in constructing strains with isogenic backgrounds. Specifically, available mutations in protease genes (16) blocks in metabolism (13) or germination may be analyzed in isogenic strains.

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