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Transduction of Lactose Metabolism in *Streptococcus lactis* C2[1]

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Ultraviolet (UV)-induced phage lysates, from lactose-positive (*lac*$^+$) *Streptococcus lactis* C2, transduced lactose fermenting ability to *lac*$^-$ recipient cells of this organism. Although the phage titer could not be determined due to the absence of an appropriate indicator strain, the number of transductants was proportional to the amount of phage lysate added. Treatment of the lysate with deoxyribonuclease had no effect on this conversion, indicating the observed genetic change was not mediated by free deoxyribonucleic acid. When the *lac*$^+$ transductants were isolated and exposed to UV irradiation, lysates with higher transducing ability were obtained. The transducing ability of this lysate was about 100-fold higher than that observed in the original lysates. The *lac*$^+$ transductants were unstable since *lac*$^-$ segregants occurred at high frequency. The phage lysate from *S. lactis* C2 also transduced maltose and mannose metabolism to the respective negative recipient cells. The results demonstrate the transduction of carbohydrate markers by a streptococcal phage and establish a genetic transfer system in group N streptococci.

Bacterial transduction is observed when a temperate bacteriophage transmits a portion of the bacterial chromosome of its host to a recipient bacterium where it may become incorporated into the chromosome (4). The occurrence of transduction has been reported in several bacterial species, but the phenomenon has not been observed in lactic streptococci by using temperate bacteriophages.

The occurrence of lactic streptococci which spontaneously, or due to induction, release bacteriophages has been reported (5, 17, 18). No published attempts were made, however, to use these temperate phages in transduction experiments. The only reported transduction research with group N streptococci was that performed by Sandine et al. (18) and Allen et al. (1). They reported transduction of tryptophan independence in *Streptococcus diacetilactis* 18-16 and streptomycin resistance from a mutant of *Streptococcus lactis* C2 to the parent strain *S. lactis* C2. However, these transduction experiments were brought about by virulent rather than by temperate bacteriophages.

During our studies on lysogeny in lactic streptococci and its possible role in lactose metabolism, *S. lactis* C2 was shown to carry a temperate phage (6). The bacteriophage was induced from this organism by exposure to ultraviolet (UV) irradiation, and its morphology was determined by electron microscopy.

The induced phage had a head approximately 40 nm in diameter and a tail length and width of about 180 nm and 6 nm, respectively. This strain has been frequently used in metabolic studies of group N streptococci without knowledge of its lysogenic nature. In this paper, we describe the ability of the temperate phage to transduce the lactose marker to lactose negative (*lac*$^-$) recipients of *S. lactis* C2. Properties of the *lac*$^+$ transductants are reported.

MATERIALS AND METHODS

Bacterial strains. All lactic streptococci used in this study are maintained in our stock culture collection. *S. lactis* C2 was maintained at −15 C in 1.0-ml portions of milk and transferred twice in lactic broth (3) before initiating an experiment. Incubation was at 32 C. The *lac*$^-$ mutants were previously isolated as spontaneous variants or by using acriflavine (7). The maltose- and mannose-negative mutants were isolated by UV mutagenesis.

Growth and induction of prophage. *S. lactis* C2 was grown in lactic broth at 32 C for 10 h. The cells were centrifuged at 5,000 × *g* for 10 min and resuspended in 0.85% saline so that a 1/20 dilution yielded an optical density of approximately 0.05 at 650 nm. A 10-ml amount of the adjusted cell suspension was transferred to a sterile glass petri dish, and the

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suspension was irradiated for 25 s during constant swirling. The UV source was a 15-W General Electric germicidal lamp held 38.5 cm above the cell suspension. The UV-treated cells were inoculated into 10 ml of broth containing 20 g of tryptone, 5 g of glucose, 4 g of NaCl, 1.5 g of sodium acetate, 150 mg of $CaCl_2 \cdot 2H_2O$, 200 mg of $MgSO_4 \cdot 7H_2O$, and 50 mg of $MnSO_4 \cdot H_2O$ per liter of deionized water. The pH of this medium was adjusted to 7.0 prior to autoclaving. Growth and lysis of the cells at 32 C were determined by measuring the change in absorbancy at 650 nm. After lysis was complete, the sample was centrifuged at 5,000 × *g* for 15 min in a Sorvall centrifuge. The supernatant fluid was filter sterilized by passing through a 0.45-μm membrane filter (Millipore Corp.) and stored at 4 C.

To prepare a concentrated phage lysate, a 600- to 1,000-ml lysate from UV-induced cultures of *S. lactis* was centrifuged and filtered as above and then recentrifuged at 54,500 × *g* for 1 h in a Beckman ultracentrifuge. The supernatant fluids were discarded, and to the pellets was added about 0.2 ml of a phage diluent containing 0.05 M potassium phosphate buffer (pH 7.0), 0.1 M NaCl, 0.005 M $MgSO_4$, and 0.005% gelatin. The centrifuge tubes were gently shaken at 4 C for 3 h to resuspend the phage, and the contents were pooled. The concentrated phage was centrifuged at 5,000 × *g* for 10 min, and the supernatant was filter sterilized as described above.

Transduction. A modification of the technique of Allen et al. (1) was used to effect transduction. Recipient cells were grown in 50 ml of lactic broth at 32 C for 4 h, centrifuged at 5,000 × *g* for 10 min, and resuspended in 5 ml of 0.85% saline. One-tenth milliliter of this cell suspension was mixed with 0.1 ml of phage lysate and spread over the surface of bromocresol purple (BCP) indicator plates (8). The plates were incubated at 32 C for 48 h, and carbohydrate-positive colonies were counted. Control plates included the recipient cells without phage for spontaneous revertants and the phage lysate for sterility.

Deoxyribonuclease test. To eliminate the possibility of free deoxyribonucleic acid (DNA) being responsible for any observed genetic change, the phage lysate was treated with beef pancreas deoxyribonuclease (Sigma Chemical Co.). A stock solution of sterile deoxyribonuclease containing 2 mg/ml was prepared by dissolving the enzyme in water and filtering through a 0.45-μm membrane filter (Millipore Corp.). For the test, 0.4 ml of phage lysate was mixed with 0.1 ml of 0.05 M $MgSO_4$ and 0.1 ml of deoxyribonuclease. The tubes were incubated at 37 C for 30 min and tested for transducing ability as described above.

Examination of lac^+ clones formed by transduction. A transductant was picked and purified. This transductant was treated with UV irradiation to obtain a lysate to test for transducing ability as described above.

To examine the stability of lactose metabolism in the transductants, the organisms were streaked on BCP-lactose indicator agar. A typical lac^+ colony was transferred to a tube of lactic broth and grown at 32 C for 16 h. The culture was streaked over the surface of the indicator agar for isolation of lac^+ colonies. All plates were incubated at 32 C for 36 h. The above procedure was performed three times, and then a typical lac^+ colony was transferred to a tube of lactic broth and grown at 32 C for about 16 h. The culture was diluted and spread over the surface of the indicator agar to obtain individual colonies. The plates were incubated at 32 C for 48 h, and 10 lac^+ colonies were separately picked into tubes containing 1.0 ml of 0.85% NaCl. The tubes were mixed thoroughly, diluted, and spread over the surface of lactose indicator agar. After 48 h of incubation at 32 C, the plates were examined for lac^- colonies.

Curing of S. lactis C2. Three different techniques were used in attempts to isolate a cured strain of *S. lactis* C2. The organism was spread over the surface of lactose indicator agar plates, and the plates were exposed to UV irradiation at high dose levels. Plates showing about 100 colonies after incubation at 32 C for 30 h were replicated on lactic agar plates previously flooded with a suspension of the temperate phage. Colonies derived from cured strains of *S. lactis* C2 would lyse or show eroded edges in the presence of the phage they had lost. The second approach was to use bacteria previouly treated with acriflavine (7), and the survivors were tested for cure by the method described above. The technique of Clarke (2) was also used which involved the repeated passage of the culture in lactic broth in the presence of 10^{-2} M sodium citrate. The culture was examined at periodic intervals for the accumulation of spontaneously cured variants.

RESULTS

Transduction of lac^- S. lactis C2. The results of a transduction experiment are shown in Table 1. The phage was induced from lac^+ *S. lactis* C2 by UV irradiation and used to transduce a spontaneous lac^- derivative of this strain. No spontaneous reversion occurred during the 48-h test period; also, the phage lysate was free of cells. However, lac^+ colonies did grow on the plates containing phage lysate and lac^- cells, indicating transduction had occurred. The phage lysate induced from the lac^- mutant was ineffective in bringing about transduction. Since the number of transductants observed was low, the phage was concentrated by ultracentrifugation and again tested for transduction. Figure 1 indicates an increase in the number of transductants per 0.1 ml and that the number of transductants found was proportional to the amount of phage lysate added.

Effect of deoxyribonuclease on the observed transduction. Since the observed genetic change could have been brought about by free DNA rather than by phage, the effect of deoxyribonuclease on the transducing system was determined. Table 2 shows the results obtained by first treating the concentrated phage lysate with deoxyribonuclease. The phage preparation was active in transduction,

and incubation of this preparation with nuclease and $MgSO_4$ had no effect on its activity. This indicated the observed change from *lac*$^-$ to *lac*$^+$ cells was not mediated by free DNA and strongly supports the conclusion that the observed genetic change was due to the temperate phage.

Preparation of high transducing lysates. Morse et al. (13, 14) noted that, in a transducing system of *Escherichia coli* K-12, partially diploid clones were formed as a result of transduction. Ultraviolet induction of these heterogenotic cultures yielded lysates giving high frequency of transduction because more phage particles carried the baterial genetic material. The *lac*$^+$ transductants formed by the streptococcal bacteriophage were thus examined for heterogenotic conditions for *lac*. Figure 2 shows that the *lac*$^+$ transductants which had acquired the *lac*$^+$ genes from the wild-type donor cell were susceptible to lysis by UV irradiation. The transducing activity of UV-induced lysate of the transductant was then compared to that of a lysate from *S. lactis* C2. Table 3 shows that lysate prepared from the transductant had about 100 times the transducing ability of the original lysate.

Instability of the transductants. The high transducing ability of the induced lysate from the transductant, as contrasted with that of the original induced lysate, suggested to us that the *lac* character was being incorporated into the phage genome in a way similar to that reported for the incorporation of the *gal* character into lambda prophage of *E. coli* K-12 (13). If this were the case, then the transductant may be a *lac*$^-$/*lac*$^+$ heterogenote and possibly unstable. Table 4 shows that the transductants were unstable. Each *lac*$^+$ colony examined contained from 10 to 39% of individuals that had lost the

TABLE 1. *Transduction of S. lactis C2 (lac*$^-$*) by phage induced from the lac*$^+$ *parent culture*

Method of transduction	*lac*$^-$ cells per 0.1 ml	Amt of phage lysate (ml)	Amt of phage broth (ml)	*lac*$^+$ colonies formed per 0.1 ml[a]
Tube[b]	5.0×10^8	1.0	0	2
	3.5×10^8	0	1.0	0
Direct[c]	1.2×10^9	0.1	0	7
	1.2×10^9	0	0.1	0
	0	0.1	0	0
Direct[d]	1.2×10^9	0.1	0	0
	1.2×10^9	0	0.1	0
	0	0.1	0	0

[a] The number observed is the average from five plates; no *lac*$^+$ colonies were observed on any of the control plates.

[b] The cells (1.0 ml) and phage were mixed and incubated at 32 C for 30 min prior to plating on indicator agar.

[c] The cells and phage were placed on indicator agar and spread over the surface.

[d] The phage lysate was prepared from the *lac*$^-$ mutant.

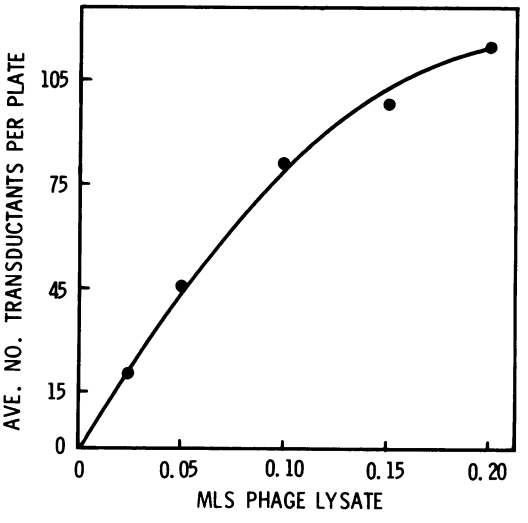

FIG. 1. *Proportionality between the number of lac*$^+$ *transductants and amount of phage lysate utilized.*

TABLE 2. *Test for effect of deoxyribonuclease on transduction of S. lactis C2*[a]

Recipient cell strain	No. of cells/ml	Amt of 0.05 M $MgSO_4$ (ml)	Amt of phage broth (ml)	Amt of phage lysate (ml)	Amt of deoxyribonuclease (2 mg/ml)	*lac*$^+$ colonies formed per 0.1 ml[b]
S. lactis C2 (*lac*$^-$)	6.9×10^9	0.1	0.5	0	0	0
S. lactis C2 (*lac*$^-$)	6.9×10^9	0.1	0.1	0.4	0	106
S. lactis C2 (*lac*$^-$)	6.9×10^9	0.1	0	0.4	0.1	99

[a] The reactants (without cells) were incubated 30 min at 37 C. Cells (0.1 ml) and 0.1 ml of reactant were added to the surface of indicator agar and spread over the surface. The concentrated phage preparation was used.

[b] The number observed is the average from four plates; no *lac*$^+$ colonies were observed on the control plates.

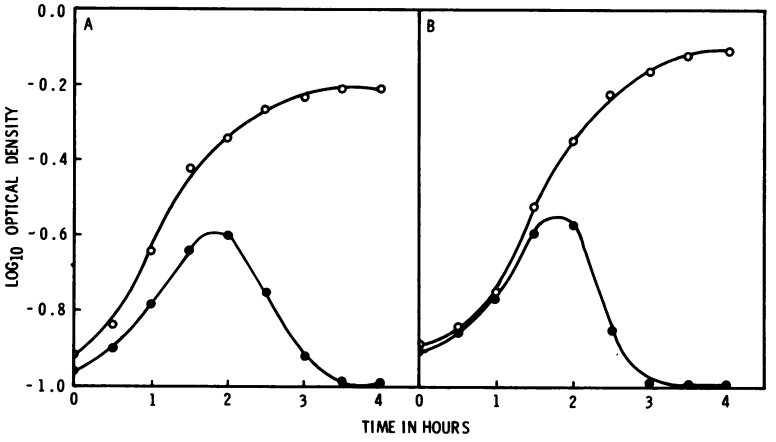

FIG. 2. *Induction of lysis in S. lactis C2 (A) and a lac⁺ transductant (B) by UV irradiation. An expotentially growing culture was harvested, washed, and exposed to UV irradiation. The irradiated suspension was then inoculated into broth, and the change in absorbancy was measured (●). Unirradiated cells served as the control (○).*

TABLE 3. *Comparison of transducing ability of lysates prepared from S. lactis C2 and a lac⁺ transductant*

Source of lysate	*lac*⁻ cells per 0.1 ml	Amt of phage lysate (ml)	Amt of phage broth (ml)	Transductants per 0.1 ml[a]
S. lactis C2	0	0.1	0.1	0
	6.5×10^8	0	0.1	0
	6.5×10^8	0.1	0	7
lac⁺ transductant	0	0.1	0.1	0
	6.5×10^8	0	0.1	0
	6.5×10^8	0.1	0	>500
	6.5×10^8	0.1[b]	0	58
	6.5×10^8	0.1[c]	0	4

[a] The number observed is the average from four plates; no *lac*⁺ colonies were observed on any of the control plates.
[b] The lysate was diluted 1/10 before using.
[c] The lysate was diluted 1/100 before using.

TABLE 4. *Occurrence of lac⁻ cells in lac⁺ transductants obtained from S. lactis* C2

Colony no.	Viable count per ml	No. colonies examined	No. *lac*⁻ colonies	*lac*⁻ colonies (%)
1	3.0×10^7	60	16	26.7
2	2.9×10^7	58	15	25.9
3	2.7×10^7	537	84	15.6
4	2.6×10^7	51	8	15.7
5	1.5×10^7	303	56	18.5
6	4.0×10^7	79	31	39.2
7	4.1×10^7	82	14	17.1
8	2.6×10^7	516	111	21.5
9	2.8×10^7	555	68	12.3
10	4.4×10^7	88	9	10.2

Lac⁺ phenotype. *S. lactis* C2 was previously reported to be unstable in lactose metabolism (9), but the frequency of *lac*⁻ occurrence was not as extensive as for the *lac*⁺ transductant.

Transduction of other markers. *S. lactis* C2 mutants unable to ferment maltose and mannose were isolated, and transduction of these markers was attempted. Table 5 shows that the phage preparation was capable of transducing the mannose and maltose markers. In contrast to the lactose transductants, neither the mannose nor the maltose transductant exhibited high-frequency transfer and neither was found to be unstable. In addition, the frequency of

TABLE 5. *Transduction of mannose- and maltose-negative cultures by phage lysates from S. lactis C2*

Recipient cells	Source of phage lysate	Transductants per 0.1 ml of lysate
S. lactis C2 Man⁻	None	0
None	*S. lactis* C2	0
S. lactis C2 Man⁻	*S. lactis* C2	190[a]
None	*man*⁺ transductant	0
S. lactis C2 Man⁻	*man*⁺ transductant	116[a]
S. lactis C2 Malt⁻	None	0
None	*S. lactis* C2	0
S. lactis C2 Malt⁻	*S. lactis* C2	51[a]
None	*malt*⁺ transductant	0
S. lactis C2 Malt⁻	*malt*⁺ transductant	45[a]

[a] The original lysates were diluted 1/10 for quantitative estimate of transductants.

transduction of the maltose and mannose genetic markers was greater than that observed with lactose metabolism.

Since the results reported here indicate a genetic system in *S. lactis* C2, we felt it was necessary to confirm our *S. lactis* C2 as a Lancefield group N streptococcus. The precipitin reaction between commerically available group N antiserum and extracts of *S. lactis* C2 was positive as reported earlier (10).

The phage lysate from *S. lactis* C2, while transducing *lac*$^-$ recipients of this strain, was unable to transfer the lactose marker to *lac*$^-$ recipients of *S. cremoris* C 11-56, B_1, and Wg_2, or *S. diacetilactis* 18-16. Exhaustive attempts to isolate an indicator or a cured strain of *S. lactis* C2 which would then serve as an indicator strain for the phage have so far been unsuccessful.

DISCUSSION

The fermentation of lactose is of obvious importance to the dairy industry, yet only recently has attention been given to lactose uptake and subsequent breakdown by lactic acid streptococci (7–10). The dairy and food industries have done little to upgrade or improve the strains on which they depend, perhaps due to the absence of a genetic system for such studies. The transducing phage reported here makes it possible for the first time to apply such studies to this group of organisms and to examine the genes controlling lactose metabolism. This phage also provides another tool for examining the causes for the spontaneous loss of lactose metabolism in lactic streptococci (7).

Previous investigators have demonstrated genetic exchange within group N lactic streptococci. Some of these results, however, were variable or could not be repeated by other research workers. Møller-Madsen and Jensen (12) reported that DNA extracted from *S. diacetilactis* and *S. lactis* var. *maltigenes* was capable of transforming the ability to ferment citrate and produce malty aroma to several *S. lactis* strains. However, attempts by Miller and Morgan (11) to effect transfer of the malty character and streptomycin resistance to selected recipient cells were unsuccessful. These authors suggested the strains examined by them were not competent. Sandine et al. (18) extracted DNA from over 40 strains of lactic streptococci in an effort to transform streptomycin resistance and tryptophan independence. No transformants were observed, although antibiotic resistance to transformable *Streptococcus* strain Challis was achieved. Perry and Slade (16) were also unsuccessful in demonstrating transformation in this group of organisms. Knittel (Ph.D. thesis, Oregon State University, Corvallis, 1965), however, observed low frequency transformation involving the Str^r and mannitol genes in *S. diacetilactis* 18-16, but results were variable in repeated experiments. Sandine et al. (18) and Allen et al. (1) described the transduction of tryptophan independence and streptomycin resistance in group N streptococci, but the transducing system involved a lytic and not a temperate phage.

In previous work (6), we observed that when a culture of *S. lactis* C2 was exposed to UV irradiation and the irradiated suspension was then incubated in broth its turbidity increased for about 2 h and then the bacteria suspension lysed as indicated by rapid clearing of the culture. This suggested that *S. lactis* C2 was lysogenic and that we had caused the induction of the prophage. Although an indicator strain could not be isolated for the proposed temperate phage, its existence was confirmed by obtaining electron micrographs which revealed its presence. In this report, we attempted to use this phage in developing a genetic transfer system in group N streptococci, and the results strongly suggest that we have discovered a transduction system. This was based on the observation that UV-induced lysates, prepared from *S. lactis* C2, "transformed" lactose-, maltose-, or mannose-negative recipient cells of this strain to the respective carbohydrate-positive phenotypes. Cell-to-cell contact was not required, ruling out conjugation as the means of genetic transfer, and treatment of the lysate with nuclease had no effect on conversion of *lac*$^-$ to *lac*$^+$ cells which indicates that the genetic change was not mediated by free DNA. The latter observation would eliminate transformation and strongly supports the conclusion that the observed genetic exchange was due to the temperate phage. Although we were unable to titer the phage due to the absence of an appropriate indicator strain, a proportionality was observed between the amount of phage lysate used and the number of *lac*$^+$ transductants obtained. This suggests it is the temperate phage responsible for the observed genetic changes and that a genetic transfer system has been discovered in group N streptococci. The high frequency lysate material prepared from the *lac*$^+$ transductant supports the conclusion that the mechanism of the genetic change observed is transduction. Whether *S. lactis* C2 harbors more than one type of prophage is unknown at present.

There are at least two different mechanisms to explain the high-frequency transduction observed in our experiments. First, analogous to

E. coli lambda *gal*$^+$ transductants (13, 14), our data support the following explanation. Upon UV induction of the prophage in the original *S. lactis* C2 *lac*$^+$ donor, a small number of phage are produced that incorporate the neighboring *lac* genes into the phage genome prior to leaving the prophage site on the bacterial chromosome. This phage then appears in the UV lysate. When this phage infects a *lac*$^-$ recipient, the bacterial chromosome carries the inserted prophage and the associated *lac*$^+$ gene in addition to its own *lac*$^-$ character. Since the *lac*$^+$ character is dominant, the recipient cell ferments lactose. This prophage-bacterium relationship would be diploid (*lac*$^-$/*lac*$^+$) for the *lac* region. Growth of this cell would then give rise to a population of cells which carries the prophage bearing the *lac*$^+$ gene in its chromosome. Induction of this population would therefore produce a very high yield of *lac*$^+$ transducing phage. This heterogenotic behavior is supported by the instability of the *lac*$^+$ character as shown by the high frequency of *lac*$^-$ cells which segregate out. Alternatively, we may be observing direct recombination between phage and bacterial genes that are ordinarily unlinked. Such high-frequency transducing derivatives of a number of generalized transducing phages have been isolated (15). It is interesting to note that, in the latter case, the bacterial genes most commonly involved are derived from extrachromosomal particles (15). This would further support the idea that lactose metabolism in lactic streptococci may be mediated by an extrachromosomal particle, as was suggested earlier (7).

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