STUDIES ON TRANSDUCTION IN LACTIC STREPTOCOCCUS ORGANISMS

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STUDIES ON TRANSDUCTION IN LACTIC STREPTOCOCCUS ORGANISMS

INTRODUCTION

Lactic streptococcus organisms consist of three species of bacteria, Streptococcus lactis, Streptococcus cremoris and Streptococcus diacetilactis, which are used by the dairy industry to manufacture numerous fermented foods. Transduction among these bacteria as a mechanism for genetic recombination has not been reported previously. Bacteriophages specific for lactic streptococci hosts, however, have for many years plagued the dairy industry by causing the lysis of these three organisms with consequent failure of a particular manufacturing operation. In view of the ubiquitousness of lactic phages, it was considered important to determine the influence these phages may have in altering the characteristics of dairy cultures.

Since transduction studies are best conducted with temperate phage mediators, it was necessary to systematically examine the genetic nature of lactic phages. For this reason, host range and lysogeny studies were undertaken. Unfortunately, a temperate phage capable of infecting both a donor and recipient strain could not be obtained; transduction studies, therefore, were conducted with virulent phages.

It was hoped that the transduction of a nutritional

requirement in S. diacetilactis and streptomycin resistance in S. lactis would serve to establish the influence of phages as mediators of genetic recombination in dairy cultures.

HISTORICAL REVIEW

Mechanisms of Bacterial Recombination

Genetic recombination, a basic requirement for variation, has been found to occur in bacteria by: sexual conjugation, the direct transfer of genetic material from a donor to recipient cell mediated by a mechanism of fusion (69, p. 141-162); episomic infection, the existence of a discrete genetic unit capable of manifesting itself as either an integrated portion of the recipient genome replicating in unison with it, or as a cytoplasmic element replicating autonomously (35, p. 67-91); transformation, "the step by step transfer of unit portions of the genetic potentialities" of donor cells to a recipient by direct transfer of deoxyribonucleic acid (DNA) without necessity of cell to cell contact (3, p. 321-335); and transduction, the transfer of genetic material from donor to recipient cell mediated by a specific bacteriophage (4, p. 408-467).

Sexual Conjugation

In 1946 Lederberg and Tatum (43, p. 113-114) reported that fully prototrophic recombinants of Escherichia coli K 12 resulted from crosses between threonine, leucine auxotrophs and biotin, methionine auxotrophs. The following year, this phenomenon was established as genetic recombination by using selective methods to eliminate parental auxotrophs (63, p. 673-684). This sexual form

of recombination was uniquely characterized by (a) the participation of the entire cell of both parents (19, p. 507-508), (b) the transfer of many characters at the same time (28, p. 75-93), and (c) the one way transfer of genetic material from a donor carrying a sex fertility factor (F^+) to an acceptor lacking the sex fertility factor (F^-) (28, p. 75-93). With the discovery by Cavalli (2, p. ix-xlvi) in 1950 of a high frequency recombinant donor capable of donating genetic material to an F^- recipient without confering upon it the donor ability, the nature of bacterial chromosomes lay open to exploration. Using the high frequency recombinant mutant (Hfr), Jacob and Wollman (69, p. 141-162) determined that the Hfr male donated its chromosome to the F^- female in a linear manner. They also found, in studies on zygotic induction(2, p. ix-xlvi), that partial chromosomal transfer was the general rule in conjugation. Having established and defined the phenomenon in *E. coli* K 12, recombination studies were extended to other strains of *E. coli* as well as to bacteria of the *Shigella*, *Pseudomonas*, *Bacillus* (26, p. 408-467) *Salmonella* and *Serratia* (9, p. 29-39) genera.

Episomic Infection

It was found by Cavalli *et al.* (17, p. 89-103) that the sex factor of *E. coli* K 12 could be transmitted during conjugation. Furthermore, it did not segregate, and was

found in all the progeny of the original zygote (37, p. 398-400). The F^+ sex factor, therefore, appeared to multiply autonomously. The Hfr isolate, however, did not exhibit autonomous replication, but was found integrated and localized on the bacterial chromosome (37, p. 398-400). The ability of the sex factor to exist as either a cytoplasmic element or in an integrated form catagorized it as an "episome", as defined by Jacob (37, p. 398-400). In 1961, Jacob and Adelberg (34, p. 669-678) pointed out the hereditary significance of episomic transfer during conjugation, noting that the chromosomal genes could be carried by the F factor. They termed this phenomenon "sex duction".

Another episomic character capable of exerting an hereditable influence is the colicin factor in enteric bacteria as described by Fredericq in 1948 (35, p. 67-91) and Ozeki and Stocker in 1960 (35, p. 67-91). During conjugation, the gene governing colicin synthesis appeared to leave its chromosomal site, multiply autonomously, then return to its locus to segregate as part of the host genome (35, p. 67-91). Jacob and Wollman (39, p. 311-335) noted that in a sexual system, whenever a zygote gave rise to a colicinogenic progeny, all the progeny were found to carry the colicinogenic character, that is, the factor did not appear to segregate. However, these same authors reported instances in which an episome in the integrated

state prevented the autonomous replication of the same element in the non-integrated state (37, p. 398-400). Such a case is represented by lysogeny (37, p. 398-400), which was defined by Lederberg as the "regular and persistent transmission of virus potentiality during the multiplication of a bacterium without overt lysis" (42, p. 51-64). Lysogeny was first identified in 1921 by Bordet and Ciuca (16, p. 365-380) who observed that filtrates of certain bacteria contained an agent capable of lysing closely related indicator strains. A rapid method for the identification of lysogenic bacteria was reported by Burnet and Lush (14, p. 27-38); lysogenic colonies were observed in plaques on an agar plate when virulent phage was spread over a pre-seeded, mixed, lysogenic, susceptible indicator. Fisk (21, p. 153-160) also reported areas of lysis on agar plates of lysogenic and suitable indicator strains when they were streaked against one another.

In 1929, Burnet and McKie (15, p. 277-284) established that lysogenic bacteria carried only the information necessary for phage production, and not the infective phage units. These non-infectious units were designated prophage by Lwoff and Gutman (50, p. 312-331). Lieb (47, p. 642-651) suggested that the genetic material of the infecting phage entered the bacterial host where it was maintained as a cytoplasmic particle. It subsequently

was shown by Jacob and Wollman, however, that the prophage was bound to the chromosome as a stable hereditary character (36, p. 468-499). Lwoff et al. (51, p. 332-333) found that this chromosome bound character was not liberated by lysozyme lysis; however, ultraviolet light irradiation induced the production and liberation of lytic phage particles.

According to Adams (1, p. 365-380), the most distinctive characteristic of lysogenized bacteria is their resistance to infection and lysis by homologous phage. This property, designated immunity by Jacob and Wollman (1, p. 365-380), appeared to be due to the presence of a cytoplasmic repressor which specifically inhibited one or several reactions leading to the synthesis and release of free phage. These authors found that lysogeny was widely distributed among bacterial species of the Enterobacteriaceae. Other workers have made the same observation for bacteria of the Pseudomonas, Vibrio, Corynebacterium, Staphylococcus (39, p. 311-335), Rhizobium (62, p. 455-465), and Agrobacterium (10, p. 180-187) genera. One exception, however, was recently reported by D. A. Smith (59, p. 522-523) in Pasteurella pestis. Williams has also reported an apparent absence of lysogeny in Salmonella pullorum (68, p. 458-471).

Lysogeny and Lysogenic Conversion

In 1951, Freeman (22, p. 675-688) reported a specialized form of lysogeny in Corynebacterium diphtheria in which a phage obtained from the lysate of a virulent strain converted a nontoxigenic strain to a toxigenic, phage resistant, form. Shortly thereafter, Barksdale (8, p. 202-212) noted that smooth Bacillus megatherium colonies became rough and wart-like upon lysogenization. In 1953, Groman (25, p. 320-325) established that toxigenicity and lysogenicity were acquired simultaneously in the converted Corynebacterium of Freeman. A description of this conversion process was offered by Jacob and Wollman (36, p. 468-499): "It appears that the presence in the genome of prophage not only endows the bacterium with a hereditary capacity for the production of a specific bacteriophage...but it also exerts a continuous influence on the metabolism of the host cell....These alterations in the physiology of the host depend upon the precise genetic constitution of the prophage as well as the residual genotype of the host in which it resides". In addition, Groman (24, p. 41-47) reported that modification of the host physiology appeared to be due to the addition of genetic information to the host genome, rather than substitution of a portion of the host genome, since loss of the phage resulted in loss of the converted character. Morse et al. (53, p. 142-156) in 1959 provided linkage data

showing that the galactose marker of E. coli K 12 was linked to phage lambda, thus establishing the intimate relationship of the phage and host genome in lysogenic conversion.

Transformation

In 1928, Griffith (7, p. 113-159) observed the in vivo alteration of rough, avirulent pneumococcal colonies to virulent, smooth forms. This alteration was mediated by heat-killed cells of the latter type. These findings were substantiated by the in vitro transformation of pneumococcus organisms by Dawson and Sia in 1931 (58, p. 681-699). In 1932, Alloway (4, p. 91-99) extended in vitro transformation to recipient pneumococcus cells using sterile, crude extracts of donor cells. But it remained for Avery, McLeod and McCarty (7, p. 137-158) to identify this "transforming principle" as DNA. In 1951, Hotchkiss (30, p. 457-461) showed DNA to be derived from the donor genome which modified or substituted in the DNA of the recipient cell during replication. Successful transformation required that the recipient cells be competent, that is, able to undergo transformation (32, p. 68-82). Cory and Starr (18, p. 146-150) quoting from Alexander and Leidy (3, p. 345-359), suggested that "competence may arise by mutations which introduce a specific somatic antigen that functions as receptor".

Hotchkiss (32, p. 49-55), however, provided quantitative evidence which suggested that all the cells of a population experience competence for a brief period during their life cycle, competence being merely a temporary physiological property, not the result of mutation. Cory and Starr (18, p. 146-150) in accordance with Hotchkiss, reported that the frequency of transformation may be dependent upon the concentration of specific DNA, all the cells being capable of transformation. Lerman and Tolmach (46, p. 68-82) also found that only a fraction of the incorporated DNA was functional; they suggested that DNA was first bound in a temporary state after which it was either expelled, which served to explain the low frequency of transformational events, or integrated into the recipient genome.

Transduction

In addition to transformation, genetic characters may recombine by an analogous process, transduction, at a significantly higher frequency. Generalized transduction, non-specific transduction capable of transmitting any one of a number of donor characters with an equal and low probability (1, p. 365-380), was first observed in 1951 by Lederberg *et al.* (44, p. 413-443). Transduction which results in the incorporation of the exogenote into the recipient genome, thereby giving rise to a haploid clone

of recombinant cells, has been termed complete transduction by Adelberg (2, p. ix-xlvi). Lederberg et al. (44, p. 413-443) using a lysate of Salmonella type 2A observed the first recorded complete transduction of genetic markers in recipient strain 22A. In 1952, Zinder and Lederberg (70, p. 79-99) determined that the phage contained in the lysate did, in fact, transfer the transduced characters. Shortly thereafter, Hershey and Chase (26, p. 408-467) demonstrated that the DNA of the phage head carried the transmittable character from donor to recipient. Furthermore, Kaiser and Hogness (40, p. 392-415) have isolated phage DNA and showed it capable of accomplishing transduction.

Although the early studies of Zinder and Lederberg (70, p. 679-699) indicated that a single lysate was able to transduce a number of different genetic markers in a culture of recipient bacteria, no more than one was donated to any single bacterium. However, Stocker et al. (61, p. 410-433) in 1953 working with Salmonella found double transductions occassionally to occur. Lennox working with E. coli and Shigella (45, p. 190-206) demonstrated the transduction of linked characters and Jacob (33, p. 207-220) found that a phage particle acting as a vector may transfer a piece of bacterial chromosome carrying one or more unrelated phages. Complete transduction also has been observed in Pseudomonas, Vibrio, Proteus (26, p. 408-

467) and most recently in Staphylococcus by Ritz (56, p. 1-90) working with complex loci and in Bacillus by Thorne (64, p. 106-111).

Generalized transduction with failure of the exogenote to integrate results in the exogenote being carried as a non-replicating unit and is termed "abortive transduction" (59, p. 410-433). Stocker et al. (61, p. 410-433) noted the formation of trails in soft agar when non-motile Salmonella were transduced with phage from a motile donor. They assumed that the allele for flagellation had entered the recipient but failed to be incorporated or replicated. The recipient carrying the allele phenotypically expressed the flagellated character, and at each cell division one motile cell and one non-motile cell resulted. As the motile cell moved through the soft agar, it left a trail behind composed of stationary daughter cells. This same phenomenon was found to occur in the transduction of purine auxotrophy in Salmonella typhimurium by Ozeki (54, p. 230-238) who termed the non-replicating exogenote a "supra numerary gene".

A unique form of transduction referred to as "restrictive transduction" occurs when E. coli K 12 is infected with phage lambda. Morse et al. (53, p. 148-156) reported that only the region controlling galactose fermentation was transducible by this phage. Also,

restrictive transduction is known to occur only with lysates prepared by induction of prophage. These workers found that induction of a gal^+/gal^- heterogenote, produced by restrictive transduction, resulted in a lambda lysate capable of affecting a high frequency of transduction (HFT). Arber et al. (6, p. 224-229) found that diluted HFT lysates used to transduce E. coli K 12 at a low multiplicity of infection resulted in heterogenotes which were immune, but non-lysogenic. This non-lysogenic "defective prophage" was incapable of replicating on induction, unless the cell also carried a normal prophage possessing the genes necessary for phage maturation. No mechanism to explain restrictive transduction or defective phage formation has yet been offered.

Recombination of Antibiotic Characters

Having examined mechanisms for bacterial recombination, it remains to examine the application of these mechanisms to the transfer of antibiotic resistance from donor to sensitive recipient cells. As quoted from Watanabe and Fukasawa (63, p. 202-209) the transfer of drug resistance by sexual conjugation from multiple resistant E. coli to Shigella and vice versa was accomplished by Akiba in 1960. That the transfer was, in fact, by conjugation and not transformation nor transduction was evidenced by the inability of cell-free filtrates of the

resistant strain to transmit the resistant character to a sensitive strain. Watanabe and Fukasawa (65, p. 202-209) found (a) that this transfer required cell to cell contact, (b) that all the antibiotic resistant characters (streptomycin, chloramphenicol, tetracycline, and sulfonamide) were transferred together in conjugation, but segregated in transduction, and (c) that all these factors were eliminated with acridine dyes, which characteristically inhibit conjugation. Since the resistant factors replicated faster than the host genome, and the resistant factors were transferred independently of the sex factor, yet were not transferred by transformation or transduction, Watanabe and Fukasawa (65, p. 202-209) have proposed the term resistance transfer factor (RTF) for this episomal genetic unit. With reference to the genetic nature of antibiotic resistance inheritance, Cavalli (16, p. 185-206) determined, via conjugation studies, that multiple loci governed resistance to chloramphenicol and terramycin in E. coli K 12. Demerec (20, p. 5-16) however, reported that streptomycin resistance was controlled by a single locus. Blair and Carr (11, p. 984-993) have reported that the reversal of susceptibility to penicillin in staphylococci was due to lysogenic conversion of sensitive strains with phage derived from Staphylococcus aureus.

Numerous instances of transformation of antibiotic resistance can be found in the literature. In 1951, Hotchkiss (30, p. 457-461) communicated the transformation of penicillin resistance in pneumococcus; he discovered that a high level resistant donor could transform a recipient to only a low level of resistance. This upheld the theory that penicillin resistance involved mutations at a series of distinct loci each of which was transformed independently. Later, (30, p. 457-461) he extended his studies to transformation of streptomycin resistance in the same species, where it was discovered that streptomycin resistance was acquired mutationally not only in a single step, but by a stepwise mechanism also. In 1956, Hashimoto (27, p. 1-9) definitively established the single step transformation of complete streptomycin resistance, which was later termed streptomycin indifference by Watanabe and Watanabe (66 p. 16-29). Again using transformation as a tool in establishing the genetic nature of the streptomycin locus, Bryan (13, p. 461-469) indicated that multiple resistance was due to the acquisition of modifiers (the en factor). Modifiers incorporated into the transformant along with the allele for a low level of resistance, served to enhance the effect of this allele ten fold, yet when incorporated alone they had no effect on the streptomycin character of the recipient.

Having transferred streptomycin resistance by episomic infection in conjugation, Watanabe and Watanabe (67, p. 30-39) extended their studies to the transduction of resistance in Salmonella typhimurium, the organism in which Bryson et al. (66, p. 16-29) were unable to reproducibly transduce streptomycin resistance. They found that streptomycin indifference could be reproducibly transduced, while step by step, intermediate resistant transductants were extremely difficult to detect, due to the slow growth of the mutants (67, p. 30-39). Similar studies conducted by Pattee (55, p. 1-45) with Staphylococcus aureus indicated that streptomycin resistance was non-transducible, while other antibiotic resistance characters (oleandomycin, erythromycin, novobiocin, chlorotetracycline) were successfully tranduced. Furthermore, Pattee found that these multiple drug resistant characters were transduced independently. Finally, Lennox, in 1955, (45, p. 190-206) observed linked transduction of streptomycin independence and maltose fermentation in E. coli K 12.

PART I

HOST RANGE SPECIFICITY AND LYSOGENY

EXPERIMENTAL METHODS

Lactic streptococci and Host Specific Bacteriophages

Lysogenicity and host range studies were conducted with lactic streptococci obtained from a stock collection maintained in the Department of Microbiology at Oregon State University. The original sources for these organisms are indicated in the doctoral thesis of W. E. Sandine (57, p. 1-123). In addition, an auxotrophic mutant of S. diacetilactis strain 18-16, found to require L-tryptophane, and a streptomycin resistant mutant of S. lactis C_2, capable of withstanding 2000 µg per ml. of streptomycin, were obtained from W. C. Brown (12, p. 1-106). These mutants were designated S. diacetilactis 18-16T and S. lactis C_2S^r, respectively.

The bacteriophages used in host range specificity determinations specific for S. cremoris 10, S. cremoris 8, S. lactis 1-10, S. lactis 23, S. lactis E, S. lactis 1-8 and S. lactis 76, were obtained from Dr. F. J. Babel, Department of Food Science, Purdue University. Those phages specific for S. lactis C_2, S. lactis C_6, and S. lactis C_{10}, were received from Dr. E. B. Collins, Department of Dairy Industry, University of California, Davis. All

other phages used were obtained from the stock collection maintained in the Department of Microbiology at Oregon State University.

Culture Propagation

Cultures were maintained by weekly transfer at the rate of one per cent inoculum in sterile lactic broth with incubation at 30°C. for 18 to 20 hours. The organisms were stored at 4°C. between transfers. The medium was sterilized by autoclaving at 121°C. at 15 pounds per square inch of pressure for 15 minutes. Composition of lactic broth is shown in Table 1. Growth curve studies with S. diacetilactis 18-16 indicated that the organisms were in the middle of the log phase of growth in broth cultures at about 4 to 5 hours after inoculation. Consequently, when it was necessary to maintain cultures in the log phase, they were repeatedly transferred to fresh, sterile lactic broth following 4.5 hours of incubation at 30°C.

Phage Propagation and Titration

One ml. of an appropriate phage lysate was delivered into a sterile 18 mm. test tube, to which was added 0.5 ml. of a sensitive indicator bacterium, and the mixture was incubated for 15 minutes. The mixture was then inoculated into 50 ml. of lactic broth containing 2×10^{-3} M $CaCl_2$ and incubated at 30°C. for 4 hours. In a second

TABLE 1

Composition of Lactic Broth Culture Medium*

Ingredient	Grams per liter
Tryptone	10.0
Yeast extract	5.0
Glucose	5.0
Lactose	5.0
Sucrose	5.0
Nutrient gelatin	2.5
Sodium chloride	4.0
Sodium acetate	1.5
Ascorbic acid	0.5
pH was 6.8 to 7.0	

*Solid lactic agar was prepared by adding 15.0 grams of agar per liter and semi-solid lactic agar was prepared by adding 8 grams of agar per liter.

50 ml. control flask of lactic broth, 0.5 ml. of the sensitive bacterium was substituted for the phage mixture. After four hours, the phage-lysed culture, as indicated by comparison with the growing control, was adjusted to pH 7 and delivered into a 10 inch strip of 3/4 inch dialysis tubing, which had been boiled for 10 minutes in a solution of ethylenediaminetetraacetic acid (EDTA). The dialysis tubing containing the phage lysate was then placed, at room temperature, in 50 grams of polyethylene glycol 4000 (Union Carbide Chemical Company, Charlston, West Virginia) until concentrated to 1/3 volume (approximately three hours). Following concentration, the lysate was filtered through a membrane filter with an average pore diameter of 0.3 micron.

One ml. of the filtered lysate, serially diluted from 10^0 to 10^{10}, was incubated in a sterile test tube with 0.5 ml. of an appropriate indicator organism in the log phase of growth (approximately 1 x 10^8 cells per ml.) for 15 minutes. The incubation mixture was then suspended in 3 ml. of lactic semi-solid agar medium containing 2 x 10^{-3}M $CaCl_2$ and flooded over the surface of a pre-hardened lactic solid agar base. After 12 hours of incubation at 30°C., the plates were scored for plaque formation and the number of plaque-forming units (PFU) recorded as the relative titer of the phage lysate.

Host Range Experiments

Five drops of an indicator bacterium to be used in determining the host specificity of 15 bacteriophages were delivered into an 18 mm. sterile test tube to which was added 3 ml. of melted semi-solid lactic agar. Following brief manual agitation, the semi-solid mixture was flooded over the surface of a solid lactic agar plate containing 20 ml. of medium. When the semi-solid overlay had solidified, sterile 1 mm. diameter filter paper discs, which had been immersed in a phage lysate solution containing 10^7 PFU per ml., were implanted on the surface. Five filter paper discs were inoculated per plate, in a radial fashion. After 20 hours of incubation at 30°C., the plates were examined for lytic zones in the area of the disc, which served as an indication of the host's susceptibility to the phage.

Detection of Lysogenicity

A modification of the cross streak method of Fisk (18, p. 153-160) was used to detect lysogeny in suspected lysogenic strains of lactic streptococci. Log phase lactic broth cultures were streaked perpendicularly using a sterile 0.01 mm. inoculating needle, onto solid lactic agar containing 2×10^{-3} M $CaCl_2$. The streak plates were permitted to dry, inverted and incubated for 24 hours at 30°C. Following incubation, the plates were

examined and zones of lysis recorded with reference to the lysogenic strain and its specific indicator strain. To limit the range of study, four by four streaks per plate were first examined; these data provided the information necessary to conduct more controlled studies on plates of one by one reciprocal crosses. The suspected lysogenic strains, indicated by the intact arm of the cross, were then incubated in broth for 24 hours in combination with their respective indicators, as signified by the lysed arm of the cross. The cultures were then filtered through a millipore filter with an average pore diameter of 0.3 micron and the filtrates then were used in more precise "spot tests" and "phage assay tests".

Aliquots (0.1 ml.) of each filtrate were spotted on an appropriate indicator lawn using a sterile inoculating needle. Indicator lawns were seeded either with 0.1 ml. of a log phase culture, which was spread directly onto the surface of a pre-hardened lactic agar plate; or, with 1.0 ml. of a log phase culture, which had been suspended in 3 ml. of melted semi-solid lactic agar and flooded over the surface of pre-hardened lactic agar base. The spotted plates were incubated for 24 hours at 30°C. and examined for lysis in the region of the spot. Filtrates of the indicator strains were also collected and spotted as above to serve as experimental controls.

One ml. samples of each of the lysates were also incubated for 15 minutes in a sterile test tube with 0.5 ml. of a log phase specific indicator, suspended in 3 ml. of semi-solid lactic agar and flooded over the surface of solid lactic agar base. Following a 24 hour incubation period, the plates were scored for plaques.

Induction of Phage Release in Suspected Lysogenic Cultures

Independent studies were also conducted on suspected lysogenic strains using ultraviolet light irradiation to induce the release of virulent phages. A sample (0.1 ml.) of the strain to be tested was inoculated into 10 ml. of lactic broth, incubated for 4 hours at 30°C. and centrifuged at 12,000 r.p.m. for 20 minutes in a Servall SS-1 centrifuge. The precipitated cells were washed twice in 10 ml. of sterile saline and again centrifuged for 20 minutes. The washed precipitate was taken up in 10 ml. of sterile saline and irradiated with shaking in a petri dish located at a distance of 6 inches from a 30 watt General Electric Germicidal bulb emitting 12.37 ergs per $cm.^2$ per second. Aliquots (1.0 ml.) were removed after 0, 30, 60, 120, 180, and 240 seconds respectively and incubated for 10 minutes in a sterile test tube with 1.0 ml. of an appropriate susceptible host in the log phase of growth. The incubation mixture was then drawn off into 10 ml. of lactic broth containing $CaCl_2$ at

a final concentration of 2×10^{-3} M and inspected at hourly intervals for lysis. After 4 hours of incubation, $CaCl_2$ at a final concentration of 2×10^{-2} M was added, the cultures shaken vigorously for 5 minutes and filtered through a sterile Seitz filter. The filtrate (1.0 ml.) was then incubated for 15 minutes with 0.5 ml. of a sensitive indicator host, suspended in 3 ml. of lactic semi-solid agar and flooded over the surface of a prepoured solid lactic agar plate. After 24 hours of incubation, the number of plaques were scored.

To artificially establish lysogeny in sensitive strains of lactic streptococci, 1.0 ml. of a log phase sensitive culture known to contain 10^8 cells per ml. was incubated for 15 minutes with 1.0 ml. of a phage lysate known to contain 10^9 PFU per ml.; the multiplicity of infection (MOI) was 10. Following the 15 minute adsorption period, the incubation mixture was inoculated into 50 ml. of lactic broth and permitted to overgrow. An aliquot (0.5 ml.) of the resulting resistant cells was then plated out in 3 ml. of semi-solid medium with 1 ml. of the phage lysate (10^9 PFU per ml.) and resistant colonies subcultured into lactic broth. The broth cultures were then streaked on solid agar plates and discrete colonies subcultured 3 times to insure purity. The final single colony isolate was transferred into broth and 0.01 ml. of the broth culture in the log phase of growth was

then streaked against a series of proposed indicator strains following the Fisk method. Ten ml. of the broth culture in log phase were also exposed to ultraviolet light irradiation for 0, 60, 180, and 300 seconds respectively and then reinoculated and examined hourly for the occurrence of lysis.

RESULTS

Host Range Experiments

The results of the host range studies, conducted on 15 stock phage lysates containing 10^7 PFU per ml. are indicated in Table 2. The only phages exhibiting a host range beyond the strain upon which they were propagated were phages for S. cremoris strain 144F, S. cremoris strain W, S. cremoris strain 10, S. cremoris strain B, S. diacetilactis strain 18-16, S. diacetilactis strain 26-2, and S. lactis 76.

Host Modified Phage

The host range experiments on the phage propagated on S. lactis C_2 provided interesting findings. This phage, when repropagated by conventional methods in lactic broth, was found to lyse the S. lactis C_2S^s strain with repeated regularity yielding a typical phage lysate containing 10^7 PFU per ml. The phage particles obtained from such a lytic culture also were able to infect and

TABLE 2

Results of Studies to Determine the Host Ranges of Various Bacteriophages Propagated on Strains of *S. lactis*, *S. cremoris* and *S. diacetilactis*

Phage source	Host range
S. cremoris 144 F	*S. cremoris* 144 F, W, KH, 11603a, 9596, B
S. cremoris W	*S. cremoris* 144 F, W, 11603a, 9596, B
S. cremoris 10	*S. cremoris* HP, 10 *S. diacetilactis* 31-8
S. cremoris 1-10	*S. cremoris* 1-10
S. cremoris 8	*S. cremoris* 8
S. cremoris B	*S. cremoris* W, 11603a, 9596, B
S. diacetilactis 26-2	*S. cremoris* 9596 *S. diacetilactis* 6B-1, 6B-3. 31-2, 18-16, 11D-3, 31-8, 26-2, 18-16T
S. diacetilactis 18-16	*S. cremoris* 11603a, 9596 *S. diacetilactis* 18-16, 18-16T
S. lactis 23	*S. lactis* 23
S. lactis E	*S. lactis* E
S. lactis 1-8	*S. lactis* 1-8
S. lactis 76	*S. lactis* 76, 18-16
S. lactis C_2S^s	*S. lactis* C_2S^s, C_2Sr
S. lactis C_2S^r	*S. lactis* C_2S^s
S. lactis C_6	*S. lactis* C_6, E

lyse the S. lactis C_2S^r strain with the release of an equally high-titered lysate. The phage particles produced in this latter repropagation, however, were incapable of lysing the S. lactis C_2S^r host from which it had been obtained and would not produce plaques on this indicator in semi-solid overlay. Finally, the altered phage produced was found to be fully capable of reinfecting and lysing the original S. lactis C_2S^s strain and exhibited a titer of 10^6 on this host in semi-solid overlay. The particles formed in the recycling of the S. lactis C_2S^r phage through S. lactis C_2S^s host, were found again to be capable of maturation on the S. lactis C_2S^r victim. A diagramatic representation of the lytic cycle of the S. lactis C_2 bacteriophage is indicated in Figure 1.

Indication of Lysogenicity in Cross Streaks

In accordance with the data obtained in the host range experiments indicating a limited host range specificity for lactic streptococcus bacteriophages, the four by four cross streaks were conducted solely within single species. The results of these cross streaks within S. cremoris, S. lactis and S. diacetilactis species are tabulated in Tables 3, 4, and 5, respectively. In addition to confirming the evidence obtained in the four by four crosses, the one by one crosses made possible the

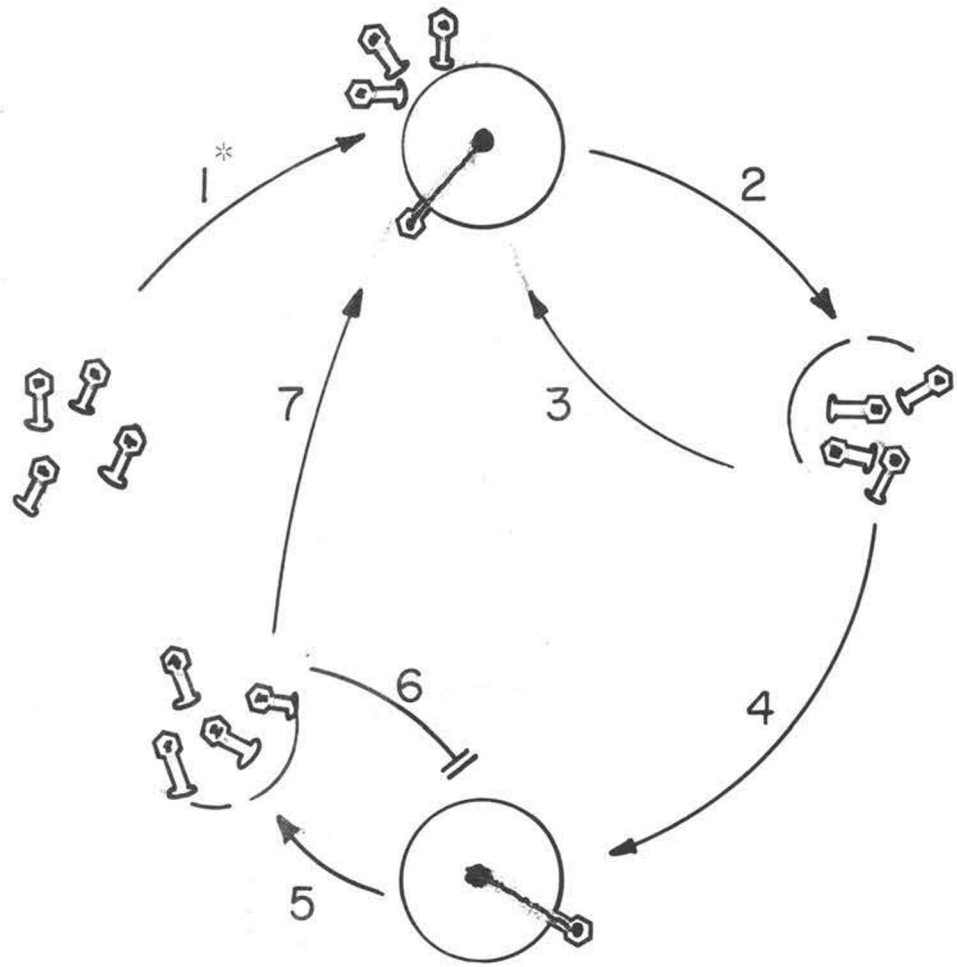

Figure 1. Lytic cycle of phage specific for S. lactis C_2.

* The numbered steps in the lytic cycle are as follows:

1. Phage derived from S. lactis strain C_2 infects S. lactis C_2S^s.
2. S. lactis C_2S^s lyses releasing infective phage particles.
3. Phage particles from S. lactis C_2S^s reinfect S. lactis C_2S^s.
4. Phage particles from S. lactis C_2S^s infect S. lactis C_2S^r.
5. S. lactis C_2S^r lyses releasing altered phage particles.
6. Altered phage particles unable to reinfect the S. lactis C_2S^r host.
7. Altered phage particles infect S. lactis C_2S^s.

TABLE 3

Results of Cross Streak Lysis Experiments
Testing for Lysogeny in Strains of *S. cremoris*

	Strain							
	144 F	W	C_3	KH	8	23	1-10	1-8
144 F	-[a]	-	+[b]	+	-	-	+	-
W	-	-	+	-	-	-	+	-
C_3	+	+	-	-	-	-	-	-
KH	+	-	-	-	-	-	-	-
8	-	-	-	-	+	-	-	+
23	-	-	-	-	-	-	-	-
1-10	+	+	-	-	-	-	-	-
1-8	-	-	-	-	+	-	-	-

[a] - indicates no evidence of lysis.

[b] + indicates lysis in the cross over area.

TABLE 4

Results of Cross Streak Lysis Experiments
Testing for Lysogeny in Strains of S. lactis

	Strain										
	27	C10	C_2S^s	E	7963	7962	1155a	11454	C_2S^r	C_6S^r	C_6S^s
27	-[a]	+[b]	-	-	-	+	-	-	+	-	-
C10	+	-	-	+	-	+	-	+	-	-	-
C_2S^s	-	-	-	-	+	+	-	-	-	-	-
E	-	+	-	-	-	-	-	-	-	-	-
7963	-	-	+	-	-	-	-	+	-	-	+
7962	+	+	+	-	+	+	-	-	-	-	-
1155a	-	-	-	-	-	-	-	-	-	+	+
11454	-	+	-	-	+	+	-	+	-	-	+
C_2S^r	+	-	-	-	-	-	-	-	-	-	-
C_6S^r	-	-	-	-	-	-	+	-	-	-	+
C_6S^s	-	-	-	-	+	+	+	+	-	+	+

[a] - indicates no evidence of lysis. [b] + indicates lysis in the cross over area.

TABLE 5

Results of Cross Streak Lysis Experiments
Testing for Lysogeny in Strains of *S. diacetilactis*

	Strain																	
	1816T	1816	DRC1	262	312	RM1	DRC2	DRC3	Da20	11D3	318	4R1	6B1	4R5	6B3	11D07	3D1	CC1
1816T	-	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
1816	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DRC1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
262	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
312	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
RM1	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
DRC2	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
DRC3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Da20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11D3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
318	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4R1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
6B1	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
4R5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6B3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
11D07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
3D1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CC1	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+

determination of the indicator bacterium for each suspected lysogenic strain. Streptococcus lactis 11454, reported to produce a bacteriocin (5, p.68), was routinely included in the cross streak test as an added control. The results of the one by one crosses are shown in Table 6. All crosses showing lytic zones in the four by four streaks listed in Tables 3, 4, and 5 but not included in Table 6 were incapable of repeating the production of a lytic area in the one by one cross. A photograph of the S. lactis 11454 control cross streaked against a susceptible indicator is represented in Figure 2. A photograph of a cross streak of 2 non-lysogenic strains is shown in Figure 3. And finally, Figures 4, 5, 6, 7, and 8 represent typical lytic crosses.

Determination of Lysogenicity Using Culture Filtrates

The nature of the lysogenic strains indicated in reciprocal cross streaks was further examined using filtrates of the lysogenic culture grown in mixed culture with the sensitive indicator bacterium. The filtrates were spotted on indicator organisms on solid, and semi-solid agar plates and the results of these examinations are shown in Tables 7 and 8 respectively. A photograph of the completely lysed spot in semi-solid agar was taken and is shown in Figure 9.

The center of the spot exhibiting complete lysis was picked and repropagation of the phage attempted on

TABLE 6

Results of One by One Cross Streaks

Cross producing lytic area	Suspected lysogenic strain	Indicator strain
S. lactis 7963 x 7962	7962	7963
S. cremoris C_3 x W	C_3	W
S. cremoris C_3 x 144 F	C_3	144 F
S. lactis C_6S^s [a] x 1155a	C_6S^s	1155a
S. diacetilactis DRC 1 x 18-16T [b]	18-16T	DRC 1
S. lactis C_2S^s x 7963	7963	C_2S^s
S. lactis 7962 x 27	?	?
S. lactis 27 x C_2S^r	C_2S^r	27
S. lactis 4R 1 x CC 1	CC 1	4R 1
S. lactis 11454 x 7963	11454	7963

[a] refers to streptomycin sensitivity, S^r indicates streptomycin resistance.

[b] T refers to tryptophane auxotrophy.

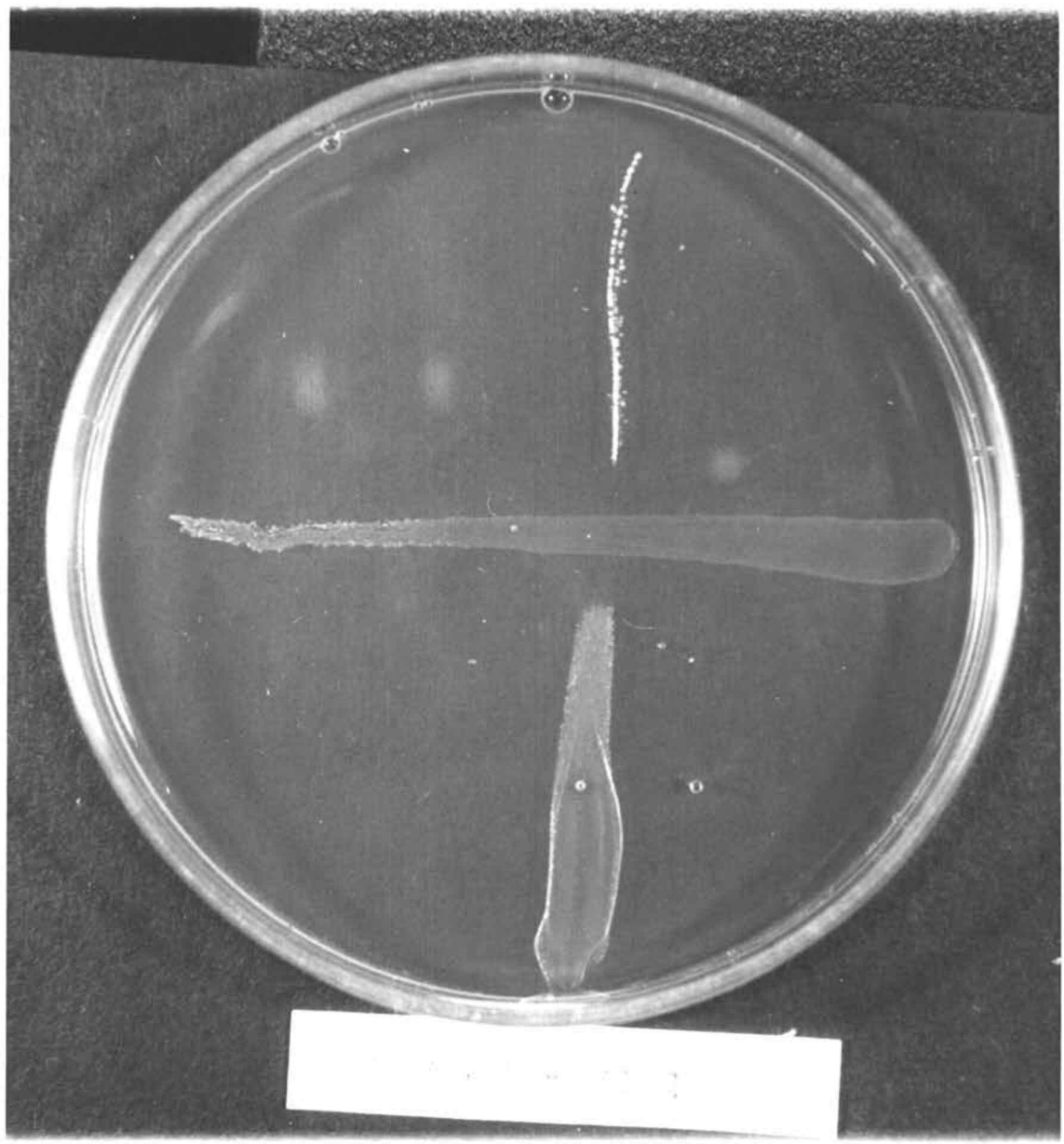

Figure 2

Lytic area produced as a result of cross streaking S. lactis 7963 (from bottom to top) against bacteriocin-producing S. lactis 11454 (from right to left).

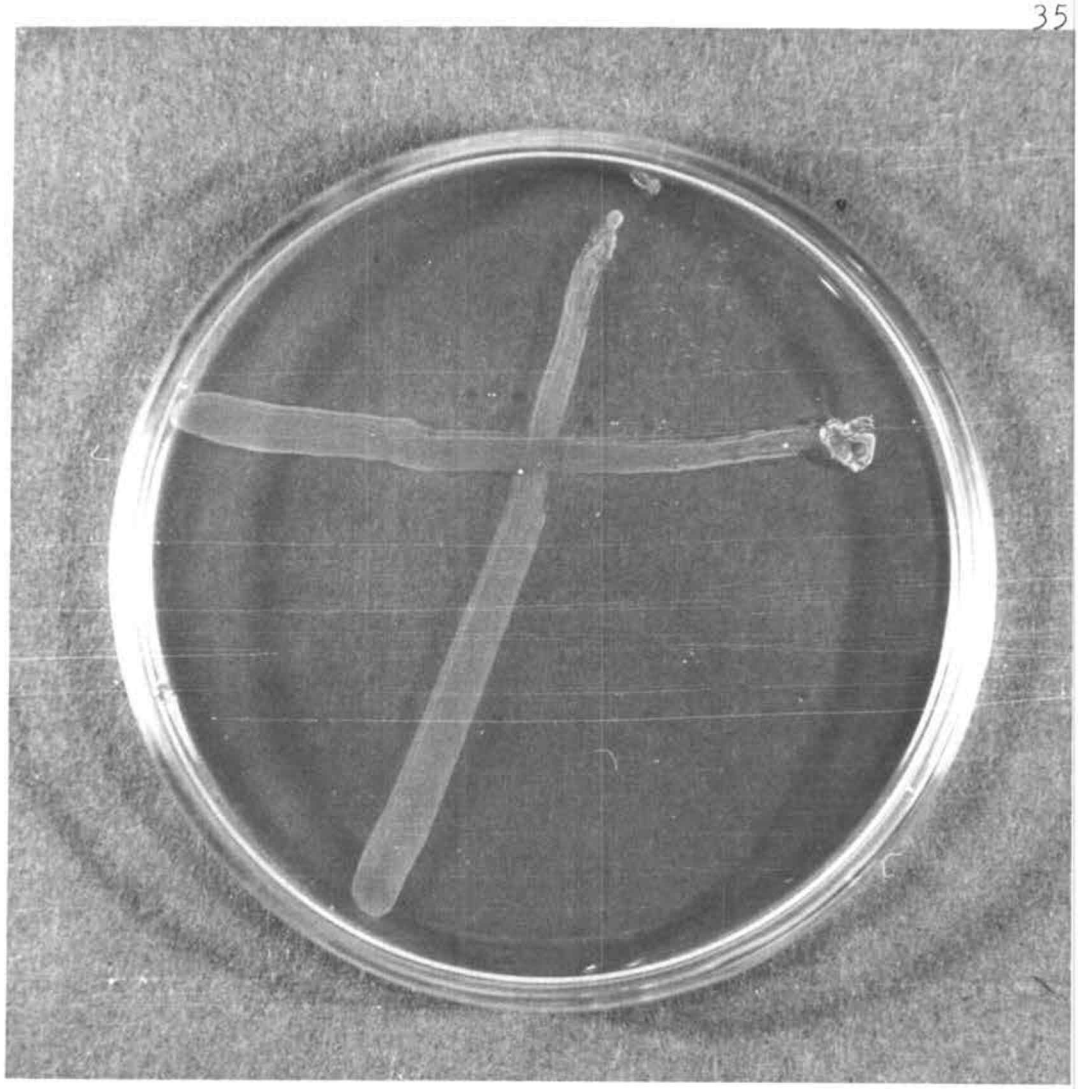

Figure 3

Results of cross streaking S. diacetilactis 18-16 (from top to bottom) and S. lactis C_2S^s (from left to right) neither of which produced bacteriocin nor was lysogenic.

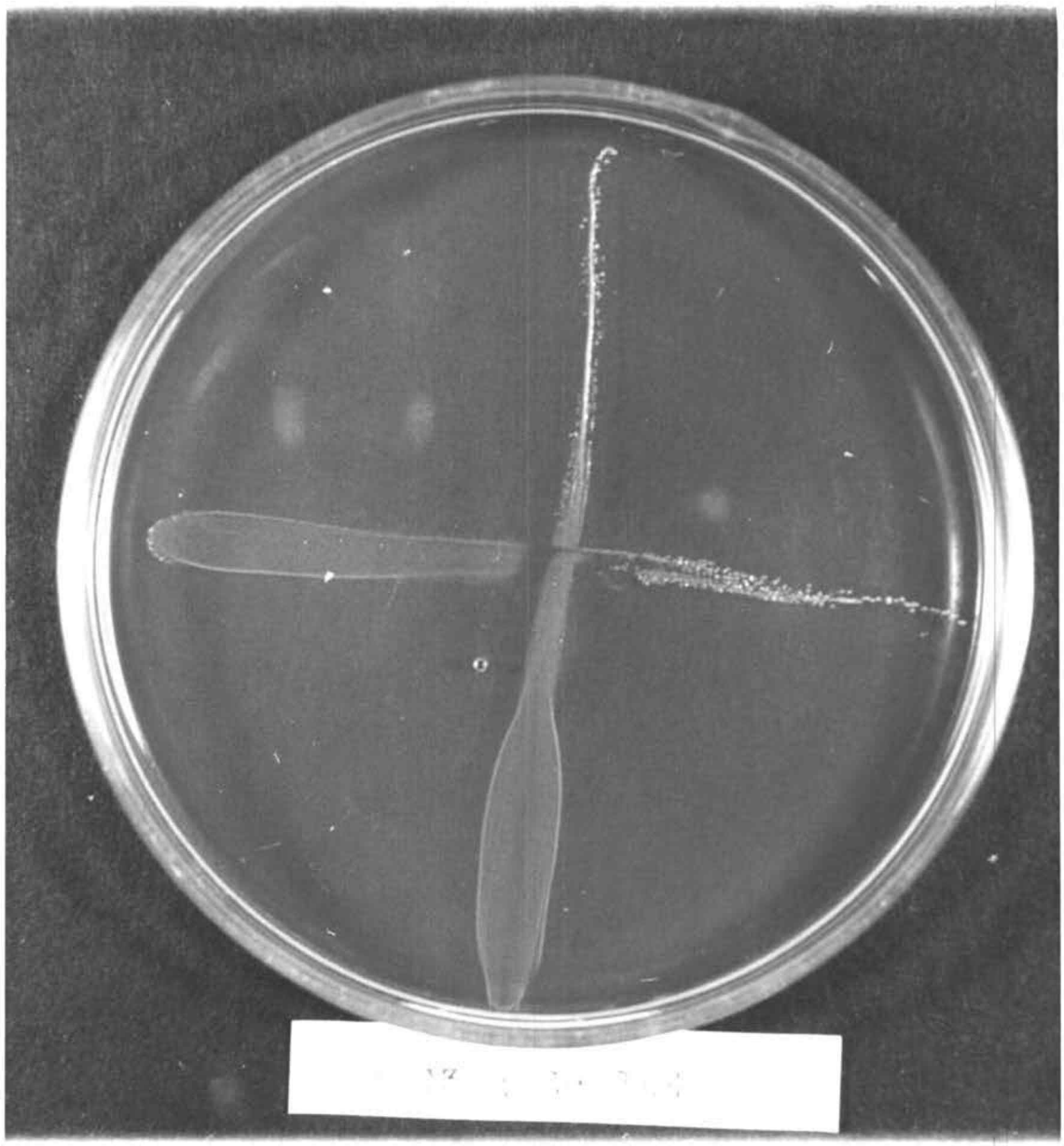

Figure 4

Evidence of bacteriophage lysis obtained when lysogenic S. cremoris C_3 (from bottom to top) was cross streaked against non-lysogenic indicator S. cremoris 144 F (from left to right).

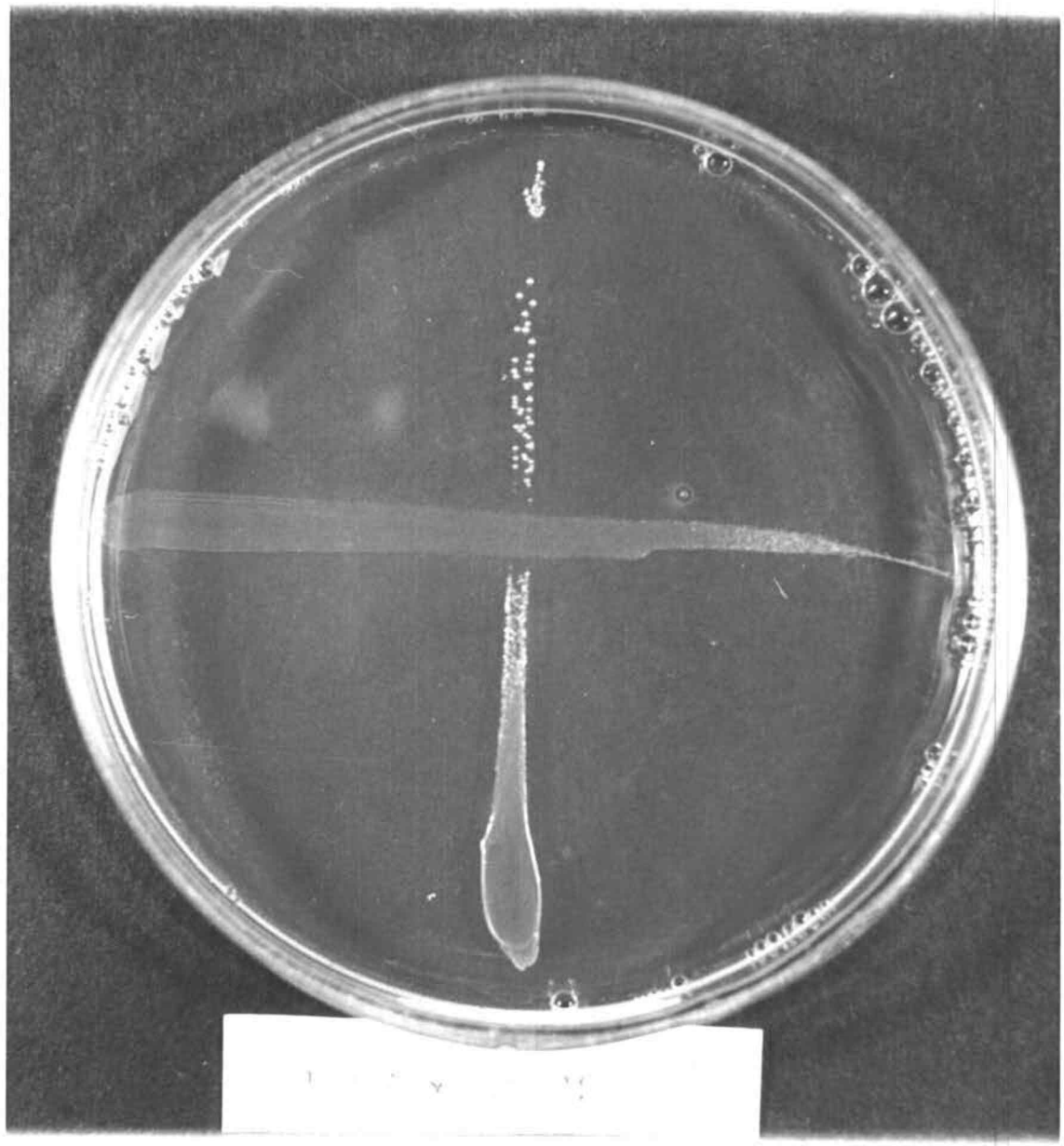

Figure 5

Evidence of bacteriophage lysis obtained when lysogenic S. lactis 27 (from left to right) was cross streaked against non-lysogenic indicator S. cremoris C_2S^r (from bottom to top).

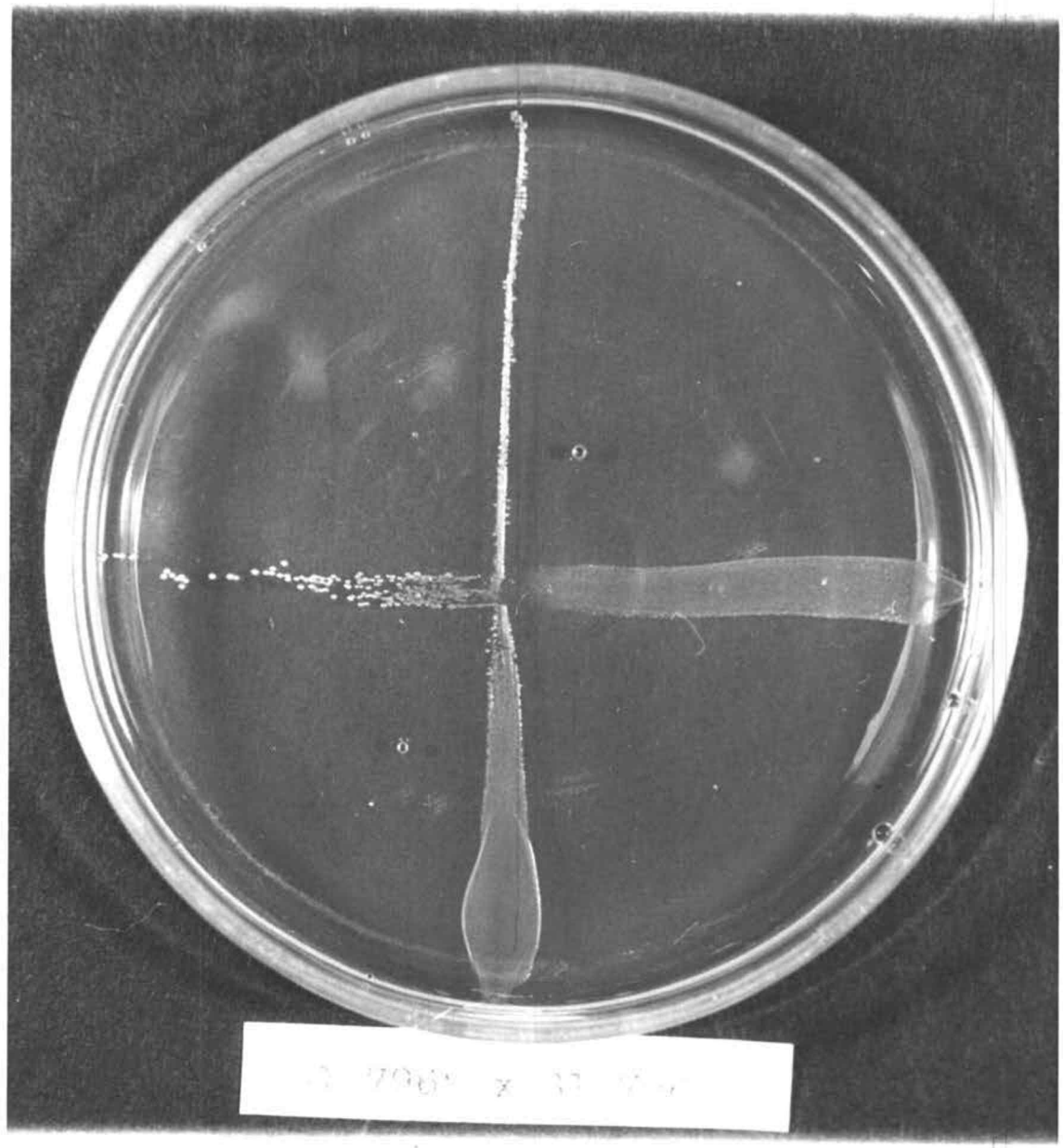

Figure 6

Evidence of bacteriophage lysis obtained when lysogenic S. lactis 7962 (from bottom to top) was cross streaked against non-lysogenic indicator S. lactis 7963 (from right to left).

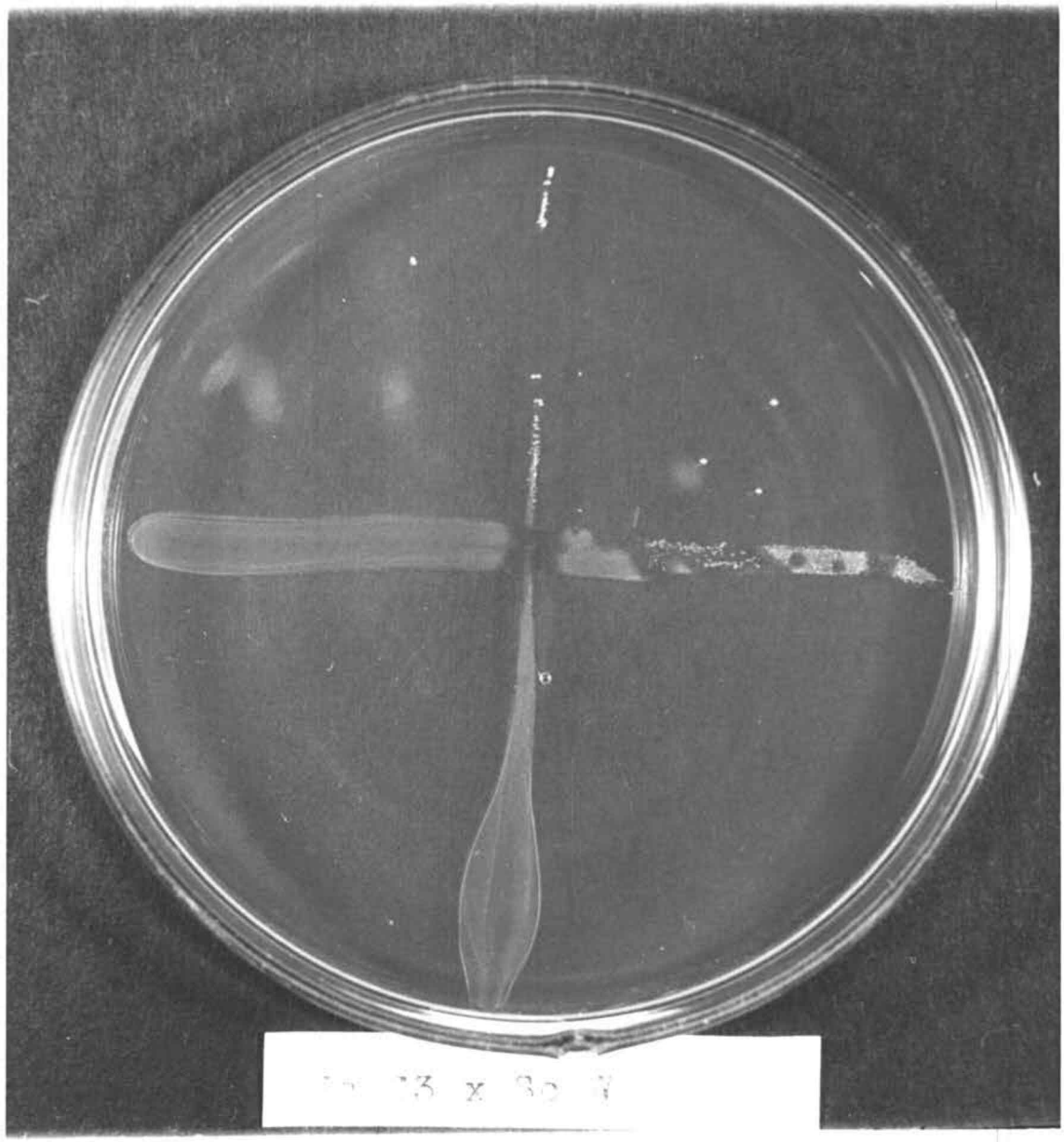

Figure 7

Evidence of bacteriophage lysis obtained when lysogenic S. cremoris C_3 (from bottom to top) was cross streaked against non-lysogenic indicator S. cremoris W (from left to right).

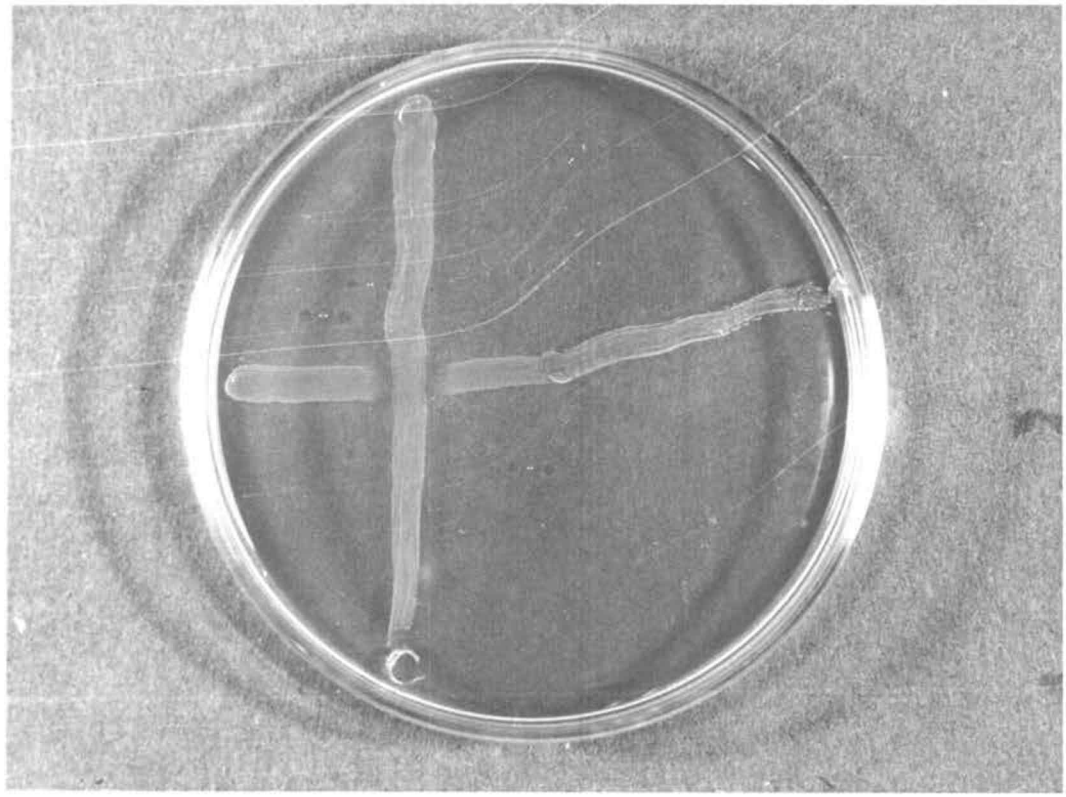

Figure 8

Evidence of bacteriophage lysis obtained when lysogenic S. lactis C_2^{Ss} (from top to bottom) was cross streaked against non-lysogenic indicator S. lactis 7963 (from left to right).

TABLE 7

Results Obtained When Mixed Strain Filtrates Were Spotted on Indicator Organisms Spread Over the Surface of a Solid Agar Plate

24-hour combined filtrate from	Indicator organism	Description of spot
S. cremoris C_3 & W	C_3	LBR [a]
S. cremoris C_3 & W	W	LBR
S. cremoris 144F & C_3	144 F	LBR
S. cremoris 144F & C_3	C_3	LBR
S. lactis 7963 & 11454	7963	NL [b]
S. lactis 7963 & 11454	11454	NL
S. diacetilactis 18-16T & DRC 1	18-16T	LBR
S. diacetilactis 18-16T & DRC 1	DRC 1	LBR
S. lactis 7963 & C_2S^s	C_2S^s	CLS [c]
S. lactis 7963 & C_2S^s	7963	NL
S. lactis 7963 & 7962	7962	NL
S. lactis 7963 & 7962	7963	NL

[a] LBR indicates lysis at the boarder of the filtrate spot.

[b] NL indicates no lysis on the plate.

[c] CLS indicates completely lysed spot.

TABLE 8

Results Obtained When Mixed Strain Filtrates Were Spotted on Indicator Organisms Suspended in Semi-solid Overlay

24-hour combined filtrate from	Indicator organism	Description of spot
S. lactis C_2S^s & 7963	C_2S^s	CLS [a]
S. lactis C_2S^s & 7963	7963	NL [b]
S. lactis 7963 & 11454	7963	NL

[a] CLS indicates completely lysed spot.

[b] NL indicates no lysis on plate.

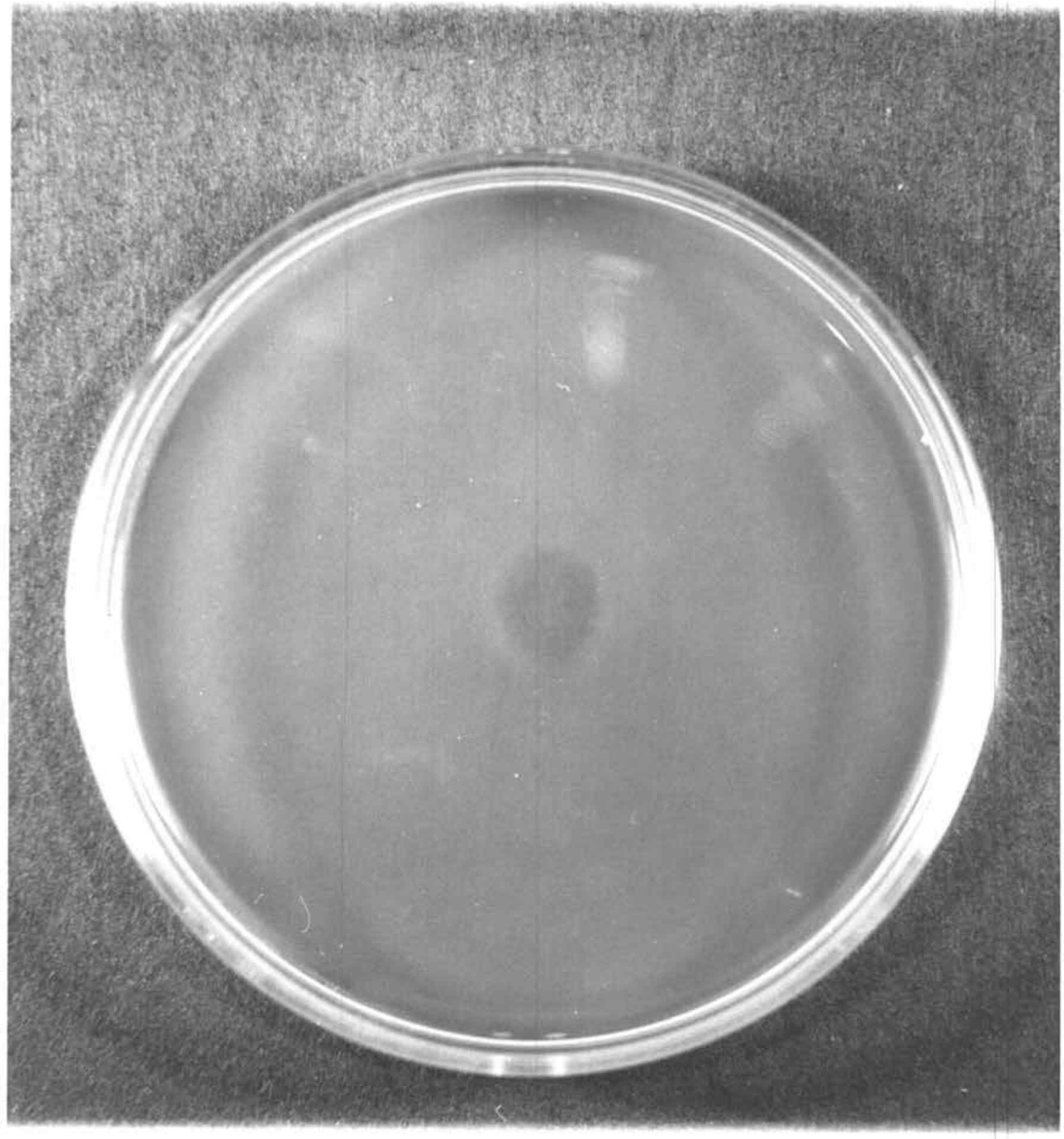

Figure 9

Lytic area resulting in semi-solid overlay agar seeded with 7963 as a result of incubation for 24 hours at 30°C. with filtrate of mixed culture of lysogenic S. lactis C_2S^s and non-lysogenic S. lactis 7963.

1.0 ml. of a log phase indicator culture diluted to 10^8, 10^7, 10^6 and 10^5 cells per ml. However, no plaque formation resulted when these filtrates in turn were spotted on semi-solid overlay agar seeded with the indicator organism (7963).

Examination of Mixed Strain Filtrates in Semi-Solid Overlay

Mixed strain filtrates were also examined directly for plaque formation by assay on semi-solid agar seeded with an appropriate indicator. Along with these were run a series of filtrates from single strain cultures to serve as controls. The results are tabulated in Tables 9 and 10 respectively.

Induction of Suspected Lysogenic Strains

Ultraviolet light irradiation studies were conducted with single strain lysogenic cultures to determine whether or not this treatment would induce the release of chromosomal prophages as free phage particles. Results of the irradiation studies are tabulated in Table 11. The plaque formed after 120 seconds irradiation of strain S. cremoris W was picked and the phage successfully re-propagated on a sensitive S. cremoris C_3 indicator strain.

Artificially Established Lysogenic Cultures

Attempts to lysogenize S. diacetilactis 18-16 and induce the prophage to produce discrete plaques, proved

TABLE 9

Results Obtained When Mixed Strain Filtrates Were Assayed on Appropriate Indicator Organisms in Semi-solid Overlay

Mixed filtrate from	Indicator organism	Number of plaques
S. cremoris 144F & C_3	144F	0
S. cremoris 144F & C_3	C_3	0
S. cremoris C_3 & W	C_3	0
S. cremoris C_3 & W	W	0
S. lactis 7963 & 11454	11454	0
S. lactis 7963 & 11454	7963	0
S. diacetilactis 18-16T & DRC 1	18-16T	0
S. diacetilactis 18-16T & DRC 1	DRC 1	0
S. lactis 7963 & 7962	7962	0
S. lactis 7963 & 7962	7963	5 (clear)

TABLE 10

Results Obtained When Single Strain Filtrates Were Assayed on Appropriate Indicator Organisms in Semi-solid Overlay

Filtrate from	Indicator	Number of plaques
S. lactis 7962	7962	0
S. lactis 7962	27	0
S. lactis 7962	7963	3 (turbid)
S. lactis CC 1	CC 1	0
S. lactis 4R 1	CC 1	0
S. lactis 4R 1	4R 1	0
S. lactis CC 1	4R 1	0

TABLE 11

Number of Plaques Formed in Semi-solid Overlay Following Ultraviolet Light Irradiation of Lysogenic Streptococci

Organism	Period of irradiation in seconds					
	0	30	60	120	180	240
S. cremoris 1-8	0	0	0	1	1	5
S. diacetilactis 26-2	0	0	2	0	1	0
S. cremoris 144F	6	1	2	0	0	2
S. diacetilactis DRC-1	0	0	1	2	2	2
S. diacetilactis 18-16T	0	-	2	4	0	3
S. lactis C_2	0	0	0	0	0	0
S. lactis E	1	4	5	2	2	0
S. lactis 27	0	0	0	0	1	1
S. diacetilactis 18-16	3	0	1	6	3	0
S. lactis C_6	0	1	1	4	7	2
S. cremoris KH	2	3	5	0	0	0
S. cremoris W	1	1	1	1	1	1
S. cremoris 10	2	0	6	5	5	0
S. cremoris 1-10	1	0	1	1	3	0
S. cremoris 8	0	0	0	0	0	0
S. cremoris 23	0	0	0	0	0	0

unsuccessful. One resistant clone, picked from a confluently lysed phage assay plate, exhibited immunity to infection by phage, and on ultraviolet irradiation for 180 seconds released plaque forming units at a very low frequency (3 PFU per 10^8 cells). A second isolated clone produced plaques in cross streaks against a sensitive 18-16 indicator, but was not immune to reinfection by homologous phage and eventually was lysed in broth culture.

DISCUSSION

Of the 15 phages examined for range of infection on 70 specific hosts, representing 3 streptococcal species, only 8 phages exhibited an extended host range. Of these only phage 10, 18-16 and 26-2 exhibited a host range encompassing two species, S. cremoris and S. diacetilactis. These results clearly indicate the limited range of host species capable of supporting the maturation of lactic phages. Since the phages proved active only within species boundaries, lysogenicity studies were limited to within each species.

The phenomenon demonstrated in the lytic cycle of the C_2 phage (Figure 1) may be explained by either of the following mechanisms. The phage obtained from infection of S. lactis C_2S^r may be a host-modified mutant incapable of reinfecting the victim. It's character,

however, may not be so altered as to render it incapable of infecting the S. lactis C_2S^s strain; following re-propagation on S. lactis C_2S^s, it looses its mutant character and returns to its former phenotype capable of lysing the S. lactis C_2S^r strain. A similar occurrence reported by Luria and Human (49, p. 557-569), has been demonstrated in Shigella dysentery virulent phage P_2, which is capable of infecting and lysing E. coli at a very low frequency. The resulting particles were found to reinfect and lyse either E. coli or Sh. dysentery with an equal and significantly higher frequency, but lost the high frequency of infection character with respect to E. coli when recycled through the Sh. dysentery host.

Secondly, this inability to reinfect the S. lactis C_2S^r host may arise from the loss of a helper phage originally carried over from the S. lactis C_2S^s host. This would assume the S. lactis C_2S^s to be lysogenic for a heterologous, perhaps defective, phage. On infection of the S. lactis C_2S^s with C_2 phage, maturation of the lysogenized prophage is accomplished and infection of S. lactis C_2S^r results from the double exposure to both phages. At this time the S. lactis C_2S^r supports only the maturation of the C_2 phage and as a consequence the helper phage is lost. Hence, the resulting lysate will be capable of infecting and lysing the S. lactis C_2S^s strain, but, due to the loss of the helper phage, will

be unable to infect and lyse the S. lactis C_2S^r strain.

In the first instance, the phage being a host-modified mutant, the alteration results from an intrisic change in the character of the phage. In the second instance, the phage existing as a symbiont with a "helper phage", the altered character of the lytic cycle results from a genetic change (the loss of prophage) in the host bacterium S. lactis C_2S^s.

Results of the one by one cross streaks indicated that either lysogenic or bacteriocin-producing bacteria were causing the lysis of an indicator strain (Table 6) in contrast to control organisms (Figure 3). Results of cross streaks using S. lactis 11454, known to be a bacteriocin producer, provided the same type of lysis that occurred when suspected lysogenic and indicator strains were streaked against one another (Figure 2). Consequently, no conclusion regarding the lysogenic nature of the intact streak could be made from these tests alone. The most definitive indication of lysogeny appeared in the cross of S. cremoris C_3 and S. cremoris W (Figure 7). It was difficult to establish, in this instance, which streak represented the indicator strain; lysis occurred at the junction around each organism. The plaque formation at the far right in the S. cremoris W streak provided presumptive evidence that strain C_3 was lysogenic for strain W. However, the opposite turned out to be the case and

these data were clarified when plaque formation was noted in semi-solid overlays of S. cremoris W cultures which had been induced to release phage by 120 seconds of irradiation under ultraviolet light. The plaques formed were picked and found to repropagate on the indicator S. cremoris C_3.

In addition to plaque formation in semi-solid on an appropriate indicator, it appeared that lysogeny could be distinguished from bacteriocin production by the filtrate spot test (Tables 7 and 8). S. lactis 11454, which was capable of producing lysis in cross streaks, did not produce a lytic area on the S. lactis 7963 indicator when a filtrate of the mixed culture was spotted. The S. lactis C_2S^s, however, did produce completely lysed spots on the S. lactis 7963 indicator when a filtrate of the mixed culture was spotted (Tables 7 and 8). The other suspected organisms were able to produce lysis at the boarder of the spot, but were considered nonlysogenic because of their inability to show complete lysis. From the data provided by the spot test, S. lactis C_2S^s was considered to be lysogenic for a phage active on indicator S. lactis 7963. In mixed culture, the phage released spontaneously from the lysogenic S. lactis C_2S^s, repropagated on indicator strain S. lactis 7963, remained in the supernatant on filtration, and produced the lysed spot on both semi-solid and solid agar seeded with the indicator.

The filtrates from a series of mixed cultures were further subjected to assay on appropriate indicators in semi-solid agar. These data (Tables 9 and 10) indicated that S. lactis strain 7962 was lysogenic for a phage active on indicator S. lactis 7963, forming clear plaques on the indicator and turbid plaques on self-infection. This technique did not provide data indicating lysogeny among any of the other organisms. It is felt, however, that this is not a conclusive finding for the absence of lysogeny in these strains. The acid-producing nature of these organisms not only limits phage titers and plaque size, but probably also limits the normal infective cycle that would follow upon the spontaneous release of a few virulent phages. Thus the clear demonstrations of lysogeny in this study (S. cremoris W for S. cremoris C_3 and S. lactis C_2S^s for S. lactis 7963) may represent cases where there is (a) the early spontaneous release of virulent phages before acid becomes limiting or (b) the release of a virulent phage at least somewhat immune to acid-inhibition of normal replication.

It was interesting to find that the irradiation of single strain cultures (Table 11), resulted in the release of mutant, virulent phages which were capable of reinfecting the lysogenic host from which they originated. These phages must represent typical host range mutants since lysogenic cultures are immune to infection by their

homologous phages. Consequently, and as was expected, the frequency of this occurrency was low, but, nevertheless, provided additional presumptive data regarding the lysogenic nature of these organisms. From these and the other data described, it may therefore be stated that a definite indication of lysogeny in lactic streptococci has been provided.

PART II

TRANSDUCTION STUDIES

EXPERIMENTAL METHODS

Donor Strains

S. diacetilactis prototroph strain 18-16 obtained from a stock collection maintained in the Department of Microbiology at Oregon State University, was used as the donor organism in the transduction of tryptophan independence. A mutant strain of S. lactis (C_2S^r) resistant to 2000 micrograms (µg) per ml. of streptomycin, obtained from W. C. Brown (12, p. 1-106), served as donor in the transduction of streptomycin resistance.

Recipient Strains

S. lactis prototroph strain C_2 sensitive to 250 ug per ml. of streptomycin (C_2S^s) obtained from the Department of Microbiology stock culture collection was used as the recipient in the transduction of streptomycin resistance. A strain of S. diacetilactis (18-16T) requiring L-tryptophan, obtained from W. C. Brown (12, p. 1-106), served as the recipient in the transduction of tryptophan independence.

Bacteriophage Mediator

The interstrain bacteriophage mediator (capable of infecting both donor and recipient organism) used in the transduction of tryptophan independence, designated phage 18-16, also was obtained from a stock collection maintained in the Department of Microbiology at Oregon State University. The interstrain bacteriophage used in the transduction of streptomycin resistance, designated phage C_2, was obtained from Dr. E. B. Collins, Department of Dairy Industry, University of California, Davis.

Progagation of Cultures

Cultures were maintained by weekly transfer of a one per cent inoculum into lactic-glucose broth with incubation at 30°C. for 18 to 20 hours. The composition of the lactic-glucose medium is shown in Table 12. In preparation for each transduction experiment, cultures were maintained in the log phase of growth by transferring 4.5-hour old cultures into fresh, sterile broth; this process was repeated at least twice before the cells were used in transduction. S. diacetilactis strains 18-16 and 18-16T were periodically screened for spontaneous mutation and reversion, respectively, by inoculation into minimal synthetic medium and minimal synthetic medium supplemented with 0.2 mg. per ml. of

TABLE 12

Composition of Lactic-Glucose Medium*

Ingredient	Grams per liter
Tryptone	20.0
Yeast extract	5.0
Glucose	10.0
Gelatin	2.5
Sodium chloride	4.0
Sodium acetate	1.5
Ascorbic acid	0.5
pH was 7.0	

*Calcium chloride at a concentration of 2×10^{-3} M was added to broth and agar media. Lactic-glucose agar was prepared by adding 15.0 grams of agar per liter and semi-solid lactic-glucose agar was prepared by adding 8.0 grams of agar per liter. The pH was adjusted with NaOH when required. Streptomycin sulfate (California Biochemical Corporation, Los Angeles, California) was added to the desired final concentration from a filter-sterilized stock solution at 10,000 µg per ml.

L-tryptophan. The composition of the minimal medium is shown in Table 13 (12, p. 1-106). The salts A and B included in this medium were prepared as shown in Table 14 according to the method of Henderson and Snell (29, p. 15-29). S. lactis strain C_2S^r and strain C_2S^s were periodically screened for reversion and spontaneous mutation of their respective streptomycin characters by inoculation into lactic-glucose broth containing streptomycin at final concentrations of 250, 500, 1000 and 2000 μg per ml.

Preparation and Assay of Transducing Phages

Phage lysates used in the transduction studies were prepared on the appropriate host organisms, S. diacetilactis strain 18-16 or S. lactis strain C_2S^r. A phage stock used as an experimental control in the transduction of streptomycin resistance was prepared on the S. lactis strain C_2S^s victim. The phage lysates were prepared in lactic-glucose broth by the method described on page 18 of this thesis. These lysates characteristically contained about 10^7 PFU per ml. The phage particles were enumerated on lactic-glucose medium in the manner described previously on page 20. The 18-16 phage prepared on the S. diacetilactis donor strain 18-16 was assayed on the donor. The C_2 phage prepared on the S. lactis recipient strain C_2S^s was assayed on the

TABLE 13

Composition of Minimal Medium[a]

Ingredient	Amount per liter
L-alanine	2.0 g.
L-arginine	2.0 g.
L-histidine	2.0 g.
L-isoleucine	2.0 g.
L-leucine	2.0 g.
L-valine	2.0 g.
L-glutamic acid	2.0 g.
L-tyrosine	2.0 g.
L-cystine	2.0 g.
L-aspartic acid	2.0 g.
L-lysine	2.0 g.
L-methionine	2.0 g.
L-phenylalanine	2.0 g.
Glucose	10.0 g.
Sodium acetate	2.0 g.
Salts A[b]	5.0 ml.
Salts B[b]	5.0 ml.
Niacin	10.0 µg.
Pyridoxamine	10.0 µg.
Calcium pantothenate	10.0 µg.
Biotin	10.0 µg.

[a] The amino acids indicated were obtained from Nutritional Biochemicals, Inc., Cleveland, Ohio.

[b] Composition of Salts A and B are indicated in Table 13.

TABLE 14

Composition of Salts A and B Used in Minimal Medium

Ingredient	Amount per 250 ml.
Salts A	
KH_2PO_4	25.0 g.
KH_2PO_4	25.0 g.
Salts B	
$MgSO_4$	10.0 g.
NaCl	0.5 g.
$FeSO_4 \cdot 7H_2O$	0.5 g.
$MnSO_4 \cdot 4H_2O$	0.5 g.
Concentrated HCl	0.5 ml.

streptomycin sensitive recipient. The C_2 phage prepared on the S. lactis donor strain C_2S^r also was assayed on the streptomycin sensitive recipient, due to its inability to form plaques on a donor host before recycling through the streptomycin sensitive host (Figure 1).

Since it is well known (52, p. 722-727) that the frequency of transduction increases with an increase in phage titer, the transducing phage preparations were concentrated; 50-ml. portions of the phage lysate were funnelled into a 12 inch strip of 3/4 inch dialysis tubing which had been previously boiled for 10 minutes in an EDTA solution. The dialysis tubing then was placed in 50 grams of polyethylene glycol 4000 (Union Carbide Chemicals Co., Charleston, W. Va.) and the lysate was permitted to concentrate to one third volume over a period of approximately three hours at room temperature. The concentrated lysate then was filtered through a presterilized (see p. 20) millipore filter with an average pore diameter of 0.3 micron. The filtered, concentrated phage preparation was again assayed for plaque-forming units per milliter and stocks exhibiting 10^9 PFU per ml. were used as "transducing phage". Stocks considered adequate were then streaked on lactic-glucose agar to insure sterility.

Transduction Technique

A modification of the precedure of Lennox (45, p. p. 190-206) was used to effect transduction. Recipient cells grown in 40 ml. of lactic-glucose broth to the log phase of growth and containing approximately 4 x 10^9 total cells, were centrifuged at 12,000 r.p.m. in a Servall SS-1 centrifuge for 20 minutes. The precipitate was suspended in 2 ml. of lactic-glucose broth to provide approximately 2 x 10^9 cells per ml. A 0.4 ml. aliquot of the suspension, containing approximately 8 x 10^8 cells was combined with 4 ml. of appropriate transducing phage containing about 10^9 PFU per ml.; this provided a final phage:host ratio of 100:1. Transduction experiments also were conducted using an identical phage preparation which had been subjected to ultraviolet light irradiation for 120 seconds under a 30 watt General Electric germicidal bulb at a distance of 6 inches. This irradiation lowered the number of infectious units to 10^7 per ml. The phage to cell ratio, however, was maintained at 100:1.

As a control to measure the spontaneous mutation rate, lactic-glucose broth was substituted in another tube in place of the phage lysate. As a control to establish the absolute necessity that transducing phage be prepared on a donor strain to bring about the transfer of streptomycin resistance, phage lysate prepared on

the S. lactis C_2S^s recipient strain was substituted in another tube for transducing phage. It should be noted that in both the tryptophan and streptomycin transductions, virulent phages were used as mediators.

Following a 30-minute period at 30°C., the transduction mixtures were centrifuged, washed twice in 5-ml. portions of lactic-glucose broth, and finally suspended in 2 ml. of the same medium. To determine the relative per cent adsorption, the supernatants from the phage-infected recipient cells were assayed for PFU and compared with the original phage titers. The bacterial kill by virulent phages also was estimated by comparison of bacterial plate counts following phage infection with the original cell count.

Screening Procedures for Recombinants

Due to the nature of the recombinants resulting from the transduction of tryptophan independence and the recombinants resulting from the transduction of antibiotic resistance, different screening procedures were employed with the two systems. The tryptophan prototrophs were screened entirely in broth, while the streptomycin resistant transductants were screened primarily on solid medium, with broth screening procedures serving merely as controls.

In the tryptophan system, 0.2 ml. aliquots from

each of the control and experimental suspensions were inoculated directly into one tube of lactic-glucose broth, one tube of minimal broth supplemented with 0.2 mg. per ml. of L-tryptophan, and eight tubes of minimal broth. Each tube contained 9.9 ml. of the appropriate medium. Following 24 hours of incubation at 30°C., the tubes were examined for growth. The lactic-glucose broth cultures then were centrifuged at 12,000 r.p.m., washed twice with 10-ml. portions of saline, and resuspended in 2 ml. of saline. Aliquots (0.2 ml.) from the saline suspension then were inoculated into one tube of lactic-glucose broth, and six tubes of minimal medium. The cultures were incubated for 12 hours at 30°C. and examined for growth.

Preliminary experiments with the streptomycin system were conducted in broth as described above. Aliquots (0.2 ml.) from each of the control and experimental suspensions were inoculated directly into one tube of lactic-glucose broth and six tubes of the same medium supplemented with 250 µg. per ml. of streptomycin. Following the 24 hour incubation period at 30°C., the growing broth cultures were used to inoculate eight tubes of lactic-glucose broth containing 250 µg. per ml. of streptomycin.

More refined screening procedures evolved with time, and an agar medium was substituted for the broth.

Aliquots (0.2 ml.) from the control and experimental suspensions were inoculated into one tube of lactic-glucose broth and one tube of the same medium containing 250 μg. per ml. of streptomycin. The remainder was spread over the surface of eleven previously prepared lactic-glucose agar plates; one contained 250 μg. per ml. of streptomycin and the remaining ten contained no streptomycin. Plates were incubated for 36 hours at 30°C. Following incubation, the ten lactic-glucose agar plates were replica plated according to the method of Lederberg and Lederberg (41, p. 399-406) onto pre-poured hardened lactic-glucose agar plates containing 250 μg. of streptomycin per ml. The replicated plates were incubated at 30°C. for 36 hours and observed for the development of colonies.

Preparation of Washed Agar

All agar used in the screening procedures was prepared by a modification of the method described by Ritz (56, p. 1-90). Flake agar (75 g.) was placed in a 4-liter flask, washed ten times with tap water, followed by three washes with distilled water and one each with double-distilled and double-deionized water. One-liter volumes were used in each of the water washes. The agar was permitted to settle overnight, and the excess water decanted. Then the agar was washed twice

with 500 ml. of 95 per cent C_2H_5OH, permitted to settle overnight, and the excess alcohol decanted. Remaining alcohol was evaporated by washing twice with 500 ml. volumes of acetone and filtering through a Buchner funnel under slight suction pressure. The agar was made ready for use by drying for 48 hours at 45°C.

Analysis of Streptomycin-Resistant Recombinants

Colonies growing on the replicated plates were picked into lactic-glucose broth. Following 24 hours of incubation at 30°C., transfers were made into the same broth containing 250 μg. per ml. of streptomycin. From these mature cultures (18 hours), 0.01 ml. aliquots were smeared with an inoculating needle onto a clean glass slide. After drying, the slides were Gram-stained according to Kopeloff's method (60, p. 16) and examined microscopically under an oil immersion lens. Cultures were assumed pure when Gram positive cocci typical for S. lactis were observed.

RESULTS

Transduction of Tryptophan Independence-Multiplicity of Infection Studies

Studies on the transduction of tryptophan independence in S. diacetilactis 18-16 T were conducted with a virulent phage mediator prepared on the fully prototrophic S. diacetilactis 18-16; the resulting

recombinants were screened in broth media. Failure to transduce with low multiplicity of infection using a phage lysate with a low titer was consistently found. Transduction with a high multiplicity of infection using a high-titered phage lysate proved successful. However, it was necessary that the recombinants be permitted a 24-hour delayed expression growth period in lactic-glucose broth prior to transfer into minimal medium; no growth was found in tubes of minimal medium inoculated directly following the transduction procedure. The control, which experienced identical manipulation in the absence of transducing phage, was never found to produce tryptophan independent progeny. The broth screening procedure, although adequate to qualitatively establish the success of transduction in producing prototrophic recombinants, failed to quantitatively determine the number of recombinants produced from a given population of recipients surviving phage infection. That is, the frequency of transduction could not be accurately determined, therefore, any tryptophan independent recombinants reported following the period of delayed expression were recorded as one original transductant, regardless of the actual number. A compilation of the data provided the results indicated in Table 15. All recombinant cultures were assayed with homologous phage and proved not to be immune.

TABLE 15

Results of the Transduction of Tryptophan Prototrophy in S. diacetilactis 18-16T at a High and Low Multiplicity of Infection

Number of experiment	Phage source	PFU[b] per ml.	Number of survivors per ml.	Number of prototrophs per ml.	MOI[c]	Frequency of transduction
1[a]	no phage	---	5×10^8	0	---	0 per 5×10^8
	18-16	1×10^8	3×10^5	0	1:1	0 per 3×10^5
2[a]	no phage	---	6×10^7	0	---	0 per 6×10^7
	18-16	1×10^{10}	5.2×10^6	at least 1[d]	100:1	1 per 5.2×10^6

[a] S. diacetilactis 18-16T served as the recipient strain.

[b] PFU indicates plaque-forming units.

[c] MOI indicates multiplicity of infection.

[d] While more than one prototroph may have been present, only one was recorded in the table for the reason given on page 66.

Transduction of Streptomycin Resistance-Multiplicity of Infection Studies

Transduction of streptomycin resistance was examined chiefly in the S. lactis strain C_2S^s recipient, which has never been observed to spontaneously mutate to resistance. Preliminary experiments conducted entirely in broth provided results similar to those obtained in the transduction of tryptophan independence in S. diacetilactis 18-16T. Recombinants screened immediately following transduction in broth containing 250 µg. per ml. of streptomycin were unable to grow, while those permitted a period of time for delayed expression in lactic-glucose broth grew on repeated transfers to lactic-glucose broth containing 250 µg. per ml. of streptomycin. Again, as in the transduction of tryptophan independence, low multiplicity of infection with phage proved ineffective, while high multiplicity of infection resulted in the production of recombinant progeny. The data regarding the significance of multiplicity of infection are tabulated in Table 16.

Studies on the Delayed Expression of Streptomycin Resistant Recombinants

To determine the minimum incubation period in lactic-glucose broth free of streptomycin necessary for the expression of the transduced character, timed experiments were undertaken. Using a phage preparation

TABLE 16

Results of the Transduction of Streptomycin Resistance in S. lactis C_2S^s, at a High and Low Multiplicity of Infection

Number of experiment	Phage source	PFU[b] per ml.	Number of survivors per ml.	Number of recombinants	MOI[c]	Frequency of transduction
1[a]	C_2S^r	1×10^9	1×10^3	at least 1[d]	100:1	1 per 1×10^3
	no phage	---	1×10^8	0	---	0 per 1×10^8
2[a]	C_2S^r	5×10^9	1×10^3	0	1:1	0 per 1×10^3
	no phage	---	1×10^9	0	---	0 per 1×10^9
3[a,e]	C_2S^r	1×10^9	4×10^4	at least 1[d]	100:1	1 per 4×10^4
	no phage	---	4×10^8	0	---	0 per 4×10^8

[a] S. lactis C_2S^s was used as the recipient strain.
[b] PFU indicates plaque-forming units.
[c] MOI indicates multiplicity of infection.
[d] See footnote [d] of Table 15.
[e] The mediator phages used to obtain these data in experiment 3 were irradiated under ultraviolet light for 120 seconds to produce a more temperate transducing phage lysate.

which had been irradiated to provide a more temperate transducing phage lysate at a multiplicity of infection of 100:1, the entire 2-ml. transducing suspension was maintained in double that volume (4 ml.) of lactic-glucose broth. Periodically, 0.1 ml. aliquots were removed and spread over the surface of a pre-hardened lactic-glucose agar plate containing 250 μg. per ml. of streptomycin. The plates were examined after 7 days of incubation at 30°C. and the results are tabulated in Table 17. It may be seen that in addition to the C_2 phage lysate prepared on strain C_2S^r, a phage lysate prepared on S. lactis C_2S^s also was used as an experimental control.

Determination of Transduction Frequency

Having obtained evidence for the occurrence of transduction of streptomycin resistance in S. lactis C_2S^s, it remained to more accurately determine the frequency at which transduction occurred. This was accomplished by screening the transductants on solid lactic-glucose agar, and replica plating onto lactic-glucose agar containing 250 μg. per ml. of streptomycin. Following 36 hours of incubation at 30°C., the plates were scored for

TABLE 17

Results of Varying the Delayed Expression Incubation Time on the Frequency of Transduction of Streptomycin Resistance in S. lactis C_2

Duration of incubation in hours	Source of phage	Number of viable bacteria per ml.	Resistant recombinants per ml.	Frequency of transduction
0	None	2×10^7	0	0 per 2×10^7
	C_2S^s	1×10^2	0	0 per 1×10^2
	C_2S^r	1.2×10^3	0	0 per 1.2×10^3
2	None	1×10^6	0	0 per 1×10^6
	C_2S^s	1×10^2	0	0 per 1×10^2
	C_2S^r	1×10^4	0	0 per 1×10^4
4	None	3×10^7	0	0 per 3×10^7
	C_2S^s	1×10^2	0	0 per 1×10^2
	C_2S^r	1×10^4	0	0 per 1×10^4
6	None	2×10^6	0	0 per 2×10^6
	C_2S^s	6×10^2	0	0 per 6×10^2
	C_2S^r	1×10^4	3	1 per 3×10^3
36	None	1×10^8	0	0 per 1×10^8
	C_2S^s [a]	------	-	----------
	C_2S^r	3×10^7	1000 [b]	1 per 3×10^4

[a] C_2S^s was not examined at 36 hours.

[b] 1000 represents an estimation of the number of colonies.

recombinant clones, each of which represented an independent, initial transduction. Results of the quantitative study are shown in Table 18. Only those clones exhibiting the proper Gram stain reaction were included in the table as streptomycin resistant recombinants.

Transduction of Streptomycin Resistance in Other Strains

Transduction studies conducted with S. diacetilactis 31-2 S^S and S. diacetilactis 6B-1 S^S gave erratic results and were abandoned when these strains were found to exhibit a high rate of spontaneous mutation to streptomycin resistance.

DISCUSSION

Under the conditions described above, it may be stated that transduction in S. diacetilactis 18-16 and S. lactis C_2 served as a mechanism for genetic recombination. Contrary to the results obtained by Morse (52, p. 722-727), using a temperate phage mediator, the virulent phage mediator used in these studies proved to be ineffective at a low multiplicity of infection in transduction of both tryptophan independence and streptomycin resistance (Tables 15 and 16). It was also found that the lytic action of the high-titered phage lysate used in the transduction of streptomycin resistance could be minimized, without destroying transducing activity, by ultraviolet

TABLE 18

Frequency of Transduction of Streptomycin Resistance in *S. lactis* C_2S^s as Determined by the Replica Plate Technique

Number of experiment	Phage source	PFU[b] per ml.	Number of survivors per ml.	Average number of colonies per master plate	Average number of recombinants per replica plate	MOI[c]	Frequency of transduction[d]
1[a]	C_2S^r	1×10^9	8×10^2	186	6	100:1	1 per 2.7×10^1
	no phage	---	1×10^8	TNTC[e]	0	---	0 per 1×10^8
2[a,f]	C_2S^r	1×10^9	4×10^3	TNTC	5.3	100:1	1 per 1.5×10^2
	no phage	---	2×10^8	TNTC	0	---	0 per 2×10^8

[a] *S. lactis* C_2S^s served as the recipient strain.

[b] PFU indicates plaque-forming units.

[c] MOI indicates multiplicity of infection.

[d] The frequency of transduction was derived by multiplying the average number of recombinants by 5, to obtain the value in terms of one ml., and dividing into the number of survivors.

[e] TNTC indicates too numerous to count.

[f] See footnote [e], Table 16, for method of phage preparation used in experiment 2.

light irradiation for 120 seconds as indicated in Tables 16, 17, and 18. The 18-16 phage proved to be more temperate and did not require ultraviolet light irradiation to insure a significant number of survivors.

Having established the occurrence in broth of transduction with a virulent phage mediator at a high multiplicity of infection, it remained to determine the frequency of this occurrence. Unfortunately, screening of nutritionally independent transductants was not possible by replica plating; it was discovered that a sufficient carry over of the tryptophane required by the mutant occurred in 0.1-ml. transfers from lactic-glucose broth to minimal broth. Furthermore, this "carry over" tryptophane, after repeated subculture, continued to supply the mutant with the required nutrient. It was therefore necessary to wash the cells after growth in lactic-glucose broth prior to screening for transduction in minimal medium. For this reason, replica plating from lactic-glucose agar to minimal agar was not attempted. It should be noted that similar difficulties in screening did not exist with streptomycin resistant transductants. Replica plating from lactic-glucose agar to lactic-glucose agar containing streptomycin in no way altered the effectiveness of the screening procedure, since the character being screened, streptomycin resistance, did not exert an influence on the master plate.

These intricate screening procedures were found necessary due to the delayed expression of the recombinant character in both the tryptophane independent and streptomycin resistant transductants. This delayed expression phenomenon rendered frequency studies inapplicable in the transduction of tryptophane independence. It did not, however, hinder frequency studies on the transduction of streptomycin resistance. It was hoped that the delayed expression period for streptomycin resistance would be of such duration as to provide for screening in broth followed by direct plating onto streptomycin supplemented agar. This, however, proved impossible, when it was observed that the delayed expression period exceeded the generation time of the recipient cell (Table 17). The broth medium in the screening procedure was therefore replaced by lactic-glucose agar and the plates containing survivors served as the master plates in replication.

After establishing the occurrence and frequency of transduction to streptomycin resistance in S. lactis C_2S^s, the definition of the transduced character became of interest. Watanabe and Watanabe (66, p. 16-29), referred to one step transduction to high level resistance as "transduction to independence". Transduction of intermediate levels of resistance have been reported by these same workers. They accomplished transduction to independence with a donor mutant obtained in one step by the

exposure of a sensitive strain to a high level of streptomycin. The S. lactis C_2S^r mutant, obtained from W. C. Brown, was isolated by a stepwise procedure. Although the transductants produced from recombination with the DNA of this donor strain were routinely screened on minimal levels of streptomycin (250 µg. per ml.), random samples (at least 50 per cent) were found capable of growing on 500, 1000, and even 2000 µg per ml. of streptomycin. There has been no report in the literature of one step transduction to independence with step-wise resistant donor, but in the S. lactis C_2S^s transduction, this may have occurred. This is in accord with the observations of Hotchkiss (30, p. 457-461) and Hashimoto (27, p. 1-9) who, working with transformation, defined the double nature of the streptomycin mutant as either a single step mutation to complete resistance, or a stepwise mutation to intermediate levels of resistance. It appears that the step-wise isolate of W. C. Brown finally attained the one-step resistance mutation and therefore might be used to transduce independence as well as intermediate resistance.

That the recombination of the streptomycin character is indeed an effect of transduction is indicated in Table 17. Here a phage lysate prepared on the S. lactis C_2S^s is shown to be ineffective in transferring the recombinant character, thus indicating the necessity of preparing the

phage mediator on the resistant strain. From the well-controlled studies on transformation of the same character conducted by W. C. Brown (12, p. 1-106), it appears that transformation is not a mechanism of recombination in this organism. To conclusively demonstrate the non-transformable nature of this character, DNAse must be added to the transducing phage preparation during the transduction procedure. Such investigations are currently in progress. Extension of these studies to milk cultures also are planned to determine whether or not transduction can occur in the natural environment for these organisms.

Demonstration of transduction as a mechanism of genetic recombination in lactic streptococcus organisms is significant from both practical and theoretical standpoints. Basic genetic studies now appear feasible with these organisms using transduction techniques. This especially will be true with the discovery of temperate transducing phages for these bacteria.

The practical importance of transduction in lactic streptococci concerns variability in mixed-strain starter cultures (mixtures of S. lactis, S. cremoris, and S. diacetilactis) which are used to manufacture various fermented products in the dairy industry. In recent years, this variability in mixed cultures has been a problem of increasing concern to dairy microbiologists and dairy manufacturers. The present investigation represents the

first demonstration of genetic exchange between these organisms and therefore at the same time provides an explaination for culture variability. For example, it was shown in this study that bacteriophages homologous for S. diacetilactis 26-2 also would infect S. cremoris 9596; phages for S. diacetilactis 18-16 also would infect S. cremoris 9596 and S. cremoris 11603a. Since S. diacetilactis is a citrate fermenting organism capable of producing large amounts of carbon dioxide in milk, it may be seen that infection of the above S. cremoris organisms with a phage originating from a S. diacetilactis provides an opportunity for variability; S. cremoris could gain the ability to ferment citrate, and in a mixed strain starter culture, this would be accompanied by an increase in carbon-dioxide production.

Numerous observations have been made in the industry of the rapid acquisition of a gassy character by starter cultures. Some of these undoubtedly have occurred as a result of the infection of starter cultures containing S. lactis and S. cremoris with phages carrying the genetic information for citrate fermentation which was gained as a result of an experience with S. diacetilactis.

Lysogeny among these bacteria also is of practical importance because the organisms are grown in mixtures. The spontaneous release of phages from lysogenic organisms provides free phage particles in the culture which

may be able to lyse other strains present in the mixture. Thus the phage-protection advantage gained by growing the organisms together may soon be lost with resulting culture failure.

SUMMARY

An investigation of transduction as a possible mechanism for genetic recombination in lactic streptococcus organisms was undertaken with the following objectives:

1. To study the genetic nature of the widely distributed lactic acid bacteriophages.
2. To establish whether or not these lactic acid bacteria are lysogenic.
3. To examine the transduction of tryptophane independence in S. diacetilactis, and streptomycin resistance in S. lactis.

Host range studies using lactic phages for S. lactis, S. cremoris and S. diacetilactis revealed little infectivity across species lines. Eight phages were capable of lysing other strains within their own species but only three phages were able to infect an additional species.

It was shown in this investigation that transduction by a virulent phage at a high multiplicity of infection occurred in lactic streptococci at a frequency of approximately 1 in 10^3 cells; that the recombinant characters exhibited a delayed expression; and that streptomycin "independence" was transduced in one step.

Different types of experiments indicated rather conclusively that lysogeny occurs in streptococcus organisms

of the lactic group. These experiments included cross streaks and filtrate spot tests of lysogenic and indicator organisms. Ultraviolet light irradiation also was shown to induce the release of small numbers of mutant phages from lysogenic strains. Furthermore, one phage recovered from the filtrate of a lysogenic bacterium (S. cremoris W) was successfully repropagated on the susceptible indicator (S. cremoris C_3).

An abnormal lytic cycle was discovered when the bacteriophage for S. cremoris C_2 was used to infect the streptomycin sensitive (C_2S^s) and resistant (C_2S^r) hosts; phages present in lysates of S. cremoris C_2S^s would lyse the C_2S^r strain but could be repropagated only on the streptomycin sensitive strain. S. cremoris C_2S^s subsequently was found to be lysogenic and it was suggested that alteration in the lytic cycle of phage C_2 resulted from the release of a heterologous helper phage which was lost on infection of the S. lactis C_2S^r strain. Host range modification of the C_2 phage also was suggested as a possibility to account for this phenomenon.

The probable role of transduction and lysogeny as contributors to variability in mixed strain starter cultures was discussed.

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