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Characterization of the Temperate Bacteriophage φadh and Plasmid Transduction in *Lactobacillus acidophilus* ADH†

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Lactobacillus acidophilus **ADH is lysogenic and harbors an inducible prophage, φadh. Bacteriophage were detected in cell lysates induced by treatment with mitomycin C or UV light. Electron microscopy of lysates revealed phage particles with a hexagonal head (62 nm) and a long, noncontractile, flexible tail (398 nm) ending in at last five short fibers. Phage φadh was classified within Bradley's B1 phage group and the *Siphoviridae* family. The φadh genome is a linear double-stranded DNA molecule of 41.7 kilobase pairs with cohesive ends: a physical map of the φadh genome was constructed. A prophage-cured derivative of strain ADH, designated NCK102, was isolated from cells that survived UV exposure. NCK102 did not exhibit mitomycin C-induced lysis, but broth cultures lysed upon addition of phage. Phage φadh produced clear plaques on NCK102 in media containing 10 mM $CaCl_2$ at pH values between 5.2 and 5.5. A relysogenized derivative (NCK103) of NCK102 was isolated that exhibited mitomycin C-induced lysis and superinfection immunity to phage φadh. Hybridization experiments showed that the φadh genome was present in the ADH and NCK103 chromosomes, but absent in NCK102. These results demonstrated classic lytic and lysogenic cycles of replication for the temperate phage φadh induced from *L. acidophilus* ADH. Phage φadh also mediates transduction of plasmid DNA. Transductants of strain ADH containing pC194, pGK12, pGB354, and pVA797 were detected at frequencies in the range of 3.6×10^{-8} to 8.3×10^{-10} per PFU. Rearrangements or deletions were not detected in these plasmids as a consequence of transduction. This is the first description of plasmid transduction in the genus *Lactobacillus*.**

Gene transfer and cloning systems are rapidly being developed for *Lactobacillus* species important in food and dairy products and bioprocessing. The availability of these methods is expected to promote rapid genetic characterization of numerous species and, potentially, provide the means for directed modification of those strains important in industrial fermentations and the intestinal tract of humans and other animals. These methods have focused largely on plasmid transfer via conjugation (16, 33), transformation (31, 35), and electroporation (6, 25). However, transduction, another powerful technique for genetic characterization and gene transfer in many microorganisms, has not been exploited in *Lactobacillus* species. Tohyama et al. (39) demonstrated that *Lactobacillus salivarius* temperate bacteriophage PLS-1 mediates generalized transduction of auxotrophic markers (lysine, proline, and serine) and lactose metabolism at frequencies of 10^{-7} to 10^{-8} per PFU. However, this remains the only report of phage-mediated gene transfer in lactobacilli despite considerable work describing both temperate and lytic phages in this genus.

Lysogeny within lactobacilli was first demonstrated by Coetzee and de Klerk (9) in two strains of *L. fermentum*. Since this initial report, several studies have now established that lysogeny is widespread throughout the family *Lactobacillaceae* (8, 12, 26, 34, 44). Evidence for lysogeny was generally limited to detection of cell lysis following induction with mitomycin C (MC) and visualization of phagelike particles under the electron microscope. Plaquing or lytic activity of temperate phages was detected only in a few cases in which indicator strains were available (8, 44). Morphological characteristics, biological properties, serological relationships, and DNA homology have been determined for selected lytic and temperate phages of *L. casei*, *L. delbrueckii* subsp. *lactis*, *L. salivarius*, and *L. delbrueckii* subsp. *bulgaricus* (2, 7, 26, 34, 36, 38, 42, 44). From these studies, preliminary classifications of *Lactobacillus* phages have been proposed (1, 26, 34, 37). To date, the *L. delbrueckii* subsp. *lactis* phage LL-H (2) and *L. casei* phages PL-1 (38, 42), J-1 (19), and FSW/FSV (36) represent the most thoroughly characterized phages.

Although extensive surveys of lysogeny in several strains of *L. acidophilus* have been performed (12), temperate phage within this important species were not isolated. *L. acidophilus* ADH is a bacteriocin-producing, bile-resistant isolate of human origin that exhibits adherence to human fetal intestinal cells (21). In an effort to define and characterize phage-mediated gene transfer systems for molecular analysis of *L. acidophilus*, we initially evaluated strain ADH for lysogeny. In the present study, a temperate bacteriophage (φadh) induced from an ADH lysogen was isolated, characterized, and found to exhibit classic lytic and lysogenic cycles of replication on strain ADH. Plasmid transduction in *L. acidophilus* ADH was then demonstrated with phage φadh.

MATERIALS AND METHODS

Bacteria, phage, and plasmids. The bacteria, phage, and plasmids used in this study are listed in Table 1. Frozen stock cultures were maintained at −20°C in MRS (Difco Laboratories, Detroit, Mich.) broth (pH 6.5) with 10% glycerol. *Lactobacillus* cultures were propagated at 37°C in MRS (pH 6.5) or BHI^+ medium (pH 7.1), composed of brain

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TABLE 1. Bacteria and plasmids

Bacteria or plasmid	Relevant characteristics[a]	Origin or reference
Bacterial strains		
L. acidophilus ADH		
NCK97	φadh$^+$, pTRK15	Derivative of NCK100 that does not show MC induction
NCK98	φadh$^-$, pTRK15, pGK12	Klaenhammer[b]
NCK100	φadh$^+$, pTRK15	Parent ADH strain; 21, 25
NCK101	φadh$^+$, *str-10 spc-11*, pTRK15	25
NCK102	φadh$^-$, pTRK15	φadh cured
NCK103	φadh$^+$, pTRK15	NCK102 relysogenized with φadh
NCK104	φadh$^+$, *str-10 spc-11*, pTRK15, pVA797	24a
NCK107	φadh$^+$, *str-10 spc-11*, pTRK15, pAMB1	24a
NCK111	φadh$^+$, *str-10 spc-11*, pTRK15, pGK12	25
NCK114	φadh$^+$, *str-10 spc-11*, pTRK15, pC194	25
NCK115	φadh$^+$, *str-10 spc-11*, pTRK15, pGB354	25
NCK116	φadh$^+$, *str-10 spc-11*, pTRK15, pGKV1	25
NCK117	φadh$^+$, *str-10 spc-11*, pTRK15, pSA3	25
L. delbrueckii subsp. *lactis*		
LKT	Indicator for phage LL-H	2
CNRZ 326	Indicator for phages LL-H and 0448	8
L. delbrueckii subsp. *bulgaricus* LT4	Indicator for phage C5	8
Plasmid		
pTRK15	Cryptic plasmid in ADH, 26.5 kb	25
pAMB1	Em^r, 26.5 kb	23
pC194	Cm^r, 2.9 kb	17
pGB354	Cm^r, 6.2 kb	4
pGK12	Cm^r Em^r, 4.4 kb	22
pGKV1	Cm^r Em^r, 4.6 kb	41
pSA3	Cm^r Em^r, 10.2 kb	11
pVA797	Cm^r, 30.7 kb	15

[a] φadh$^+$, φadh lysogen; φadh$^-$, cured of the φadh prophage; *str-10*, streptomycin resistance (1 mg/ml); *spc-11*, spectinomycin resistance (300 μg/ml); Cm^r, chloramphenicol resistance; Em^r, erythromycin resistance; kb, kilobases.

[b] NCK Culture Collection of T. R. Klaenhammer, North Carolina State University, Raleigh.

heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 0.5% yeast extract (BBL), 0.7% glucose, and 0.1% Tween 80 (Sigma Chemical Co., St. Louis, Mo.). In agar media, BBL agar was used at 1.5%. Soft-agar overlays contained 0.6% agar. Agar media used for phage assays were MRS, BHI$^+$, *Lactobacillus* selection (LBS) (pH 5.5; BBL) supplemented with 10 mM $CaCl_2$, and MRT (32) (pH 6.0) supplemented with 0.1% Tween 80. For LBS and MRT agars, $CaCl_2$ was added before autoclaving. For MRS and BHI$^+$, a sterile solution of $CaCl_2$ was added to presterilized media tempered at 45°C.

Induction of φadh. (i) MC induction. *L. acidophilus* ADH was propagated in MRS broth to an optical density at 590 nm (OD_{590}) of 0.3 to 0.35. The cells were harvested by centrifugation (10,410 × *g*, 8 min, 4°C) and suspended in fresh MRS broth to a final OD_{590} of 0.1. MC (Sigma) was added to a final concentration of 0.1 to 0.5 μg/ml, and incubation continued at 37°C until cell lysis was complete.

(ii) UV induction. Cells from an overnight (about 10 h) culture of *L. acidophilus* ADH in BHI$^+$ broth were harvested, washed twice with 0.85% NaCl, and suspended to the original culture volume in 0.85% NaCl. Ten-milliliter portions were placed in glass petri dishes (100 by 15 mm) and irradiated with long-wave UV light for 15 s. The UV source was two General Electric G15T8 germicidal lamps (15 Wa) placed 25 cm above the petri dish. The dish was rotated slowly during the exposure time. The UV-irradiated cells were diluted in BHI$^+$ broth to an OD_{590} of 0.1 and incubated at 37°C until lysis was complete.

Isolation of a prophage-cured derivative of strain ADH. Cells of *L. acidophilus* ADH were UV irradiated as described above. Immediately after treatment, the cells were diluted in 10% BHI$^+$ broth, plated on BHI$^+$ agar, and incubated aerobically for 24 h at 37°C. Single colonies were picked to BHI$^+$ broth, incubated for 24 h, and then tested for MC induction. Cultures not induced by MC were further evaluated for sensitivity to φadh in broth and on agar (spot assay) in the presence of 10 mM $CaCl_2$. All derivatives were confirmed as *L. acidophilus* ADH by Gram stain, catalase test, carbohydrate fermentation pattern, growth characteristics at 15 and 45°C, and plasmid DNA profile (25).

Purification of φadh. A 2-liter portion of phage lysate was treated with 1 μg of DNase I (Sigma) and 1 μg of RNase A (Sigma) per ml for 30 min at 37°C. Cell debris was removed by centrifugation (6,084 × *g*, 10 min, 4°C). Phage were concentrated with 10% polyethylene glycol 8000 (J. T. Baker Chemical Co., Phillipsburg, N.J.) and 0.5 M NaCl as described by Yamamoto et al. (43). Phage particles were purified first through CsCl discontinuous density gradients (1.3, 1.5, and 1.7 g/cm^3) followed by CsCl equilibrium density gradient centrifugation (20). Discontinuous gradients were centrifuged for 2.5 h at 25,000 rpm in a Beckman SW27 rotor; equilibrium gradients were centrifuged for 17 h at 55,000 rpm in a Beckman 70.1 Ti rotor. Phage purified from CsCl gradients were dialyzed twice against 2 liters of phage buffer (20 mM NaCl, 10 mM $MgCl_2$, 20 mM Tris hydrochloride, pH 8.0).

Plaque formation assay. *L. acidophilus* NCK102 was propagated in MRS broth to an OD_{600} of 0.5. Cells were collected by centrifugation and suspended to the original volume in MRS broth (pH 5.5) plus 20 mM $CaCl_2$. Phage dilutions were made in MRS broth (pH 5.5). A 0.1-ml amount of the phage sample and 0.5 ml of cells were mixed and incubated for 15 min at 37°C. A 3-ml portion of LBS plus 10 mM $CaCl_2$ soft

agar was mixed with the phage-cell sample and layered onto prepoured LBS–10 mM $CaCl_2$ agar plates. Plates were incubated aerobically, and plaques were enumerated after 24 h at 37°C.

Isolation of phage φadh DNA. Purified phage preparations were extracted twice with an equal volume of phenol (Ultra Pure; American Research Products Co., Solon, Ohio) saturated with 50 mM Tris hydrochloride (pH 8.0). Aqueous and organic phases were separated by centrifugation. The aqueous phase was reextracted twice with an equal volume of chloroform-isoamyl alcohol (24:1, vol/vol). Phage DNA was precipitated at −20°C overnight following addition of 0.1 volume of 3 M sodium acetate and 2 volumes of cold 95% ethanol. The DNA was pelleted by centrifugation (16,000 × *g*, 10 min, 4°C) and suspended in 1.5 ml of 10 mM Tris hydrochloride (pH 7.9)–10 mM NaCl–10 mM $MgCl_2$. Phage DNA was dialyzed for 24 h against 2 liters of the same buffer and stored at 4°C. The DNA concentration was determined by A_{260} (24a).

Restriction enzyme analysis. Restriction enzymes purchased from Bethesda Research Laboratories, Gaithersburg, Md., and International Biotechnologies, Inc., New Haven, Conn., were used as recommended by the suppliers. Determinations of cohesive ends on φadh DNA were carried out by heating restriction digests of phage DNA to 70°C for 15 min prior to electrophoresis on 0.8% agarose gels (10). Sums of the *Bgl*II, *Eco*RI, and *Cla*I restriction fragments were averaged and used to calculate the length in kilobases of φadh DNA.

DNA isolation and hybridization. Plasmid DNA was isolated and purified through CsCl-ethidium bromide density gradients (25). *Lactobacillus* chromosomal DNA was prepared as described previously (18). An electroblot apparatus (Hoefer Scientific Instruments, San Francisco, Calif.) was used to transfer *Eco*RI-digested chromosomal and bacteriophage DNAs from agarose gels to nylon membranes (Magnagraph, 0.45-μm; Micron Separations Inc., Honeoye Falls, N.Y.). Bacteriophage DNA was labeled with [^{35}S]- or [^{32}P]-dCTP by a Multiprime DNA labeling system (Amersham International, Arlington Heights, Ill.). Hybridization reactions and autoradiography were performed as described previously (24).

Electron microscopy of phage. Purified phage was absorbed on carbon-coated grids (200 mesh; 2% collodium film) and negatively stained with 2% uranyl acetate (pH 4.5). Photographs were taken with a JEOL JEM 100S electron microscope at 60 kV.

Transduction assay. Cells to be used as recipients in transduction experiments were grown in MRS broth (pH 6.5) at 37°C for 5 to 6 h to an OD_{590} of 0.4 to 0.5. The cells were harvested by centrifugation, washed with MRS broth (pH 5.5), and suspended to the original volume in MRS broth (pH 5.5) containing 20 mM $CaCl_2$. Phage used in transduction experiments were induced with 0.1 μg of MC per ml from ADH lysogens carrying the plasmid of interest and concentrated 35- to 50-fold from the lysates by precipitation with 10% polyethylene glycol 8000 plus 0.5 M NaCl. Concentrated phage preparations were suspended in phage buffer and filter sterilized (0.45 μm) prior to use in transduction assays. A 1-ml portion of recipient cells (~10^8 CFU/ml) was mixed with 1 ml of the phage suspension, and this mixture was incubated at 37°C for 2 h to allow for phenotypic expression of antibiotic resistance. A 0.2-ml amount of this mixture was spread on MRS agar supplemented with 6 μg of chloramphenicol (Cm) or 1 μg of erythromycin (Em) per ml. Plates were incubated at 37°C, anaerobically, and transductants were scored after 48 to 72 h. Experimental controls included selective plating of the following: recipient cells without phage, phage φadh without cells, and cells plus phage plus DNase (70 μg/ml) to rule out transformation. Confirmation of transductants was determined by both phenotypic expression of the plasmid-borne marker(s) and plasmid analysis.

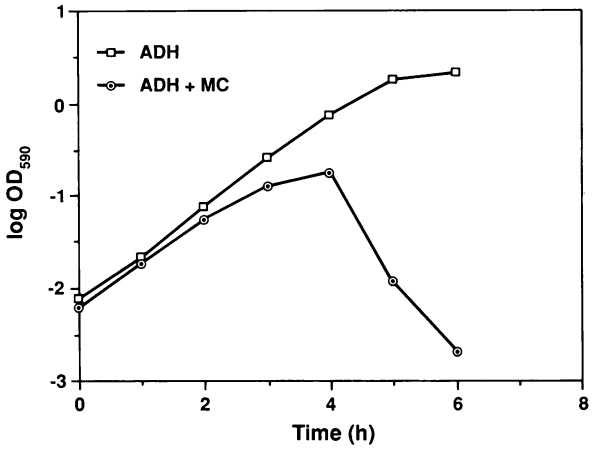

FIG. 1. Effect of MC (0.1 μg/ml) on growth of ADH cells in MRS broth, pH 6.0. Symbols: □, without MC; ⊙, with MC.

RESULTS

Induction of cell lysis. Figure 1 shows lysis of ADH cells following addition of 0.1 μg of MC per ml to a log-phase culture. Treatment of ADH cells with UV light resulted in a similar lysis curve (data not shown). The optimum conditions for inductive lysis of ADH cells occurred with 0.1 μg of MC per ml and early-log-phase cells suspended in MRS broth at a pH between 6.0 and 6.4. Under these conditions, cell lysis was observed within 4 to 5 h after addition of MC.

To determine whether active phage were produced, cell lawns of lactobacilli that are established indicator strains for *Lactobacillus* phage (2, 8) were prepared on MRS agar plus $CaCl_2$ and spotted with a sterile filtrate from an ADH lysate. No plaques or zones of inhibition were detected on *L. delbrueckii* subsp. *bulgaricus* LT4, *L. delbrueckii* subsp. *lactis* LKT, and *L. delbrueckii* subsp. *lactis* CNZ326. Attempts to demonstrate plaque formation on *L. acidophilus* ADH in MRS agar (pH 6.5) were not successful.

Characterization of φadh. Cell lysates induced from strain ADH were examined by electron microscopy for the presence of phage particles. Intact phage particles were detected (Fig. 2). These data provided evidence that phage induction by MC was responsible for lysis of ADH cells. The phage, designated φadh, has a hexagonal head (diameter, 62 nm) and a long, noncontractile, striated tail (398 nm long and 18 nm wide). No collar or tail sheath was detected. A base structure and five fibers were observed at the end of the tail.

Phage φadh DNA was extracted from purified phage particles and characterized. The phage genome is a linear double-stranded DNA molecule of 41.7 kilobases. A physical map of the φadh genome was generated by using restriction endonucleases (Fig. 3). Cohesive ends were detected at the positions indicated in Fig. 3.

Isolation and characterization of NCK102. Demonstration of phage particles following MC induction prompted experiments to further demonstrate that *L. acidophilus* ADH

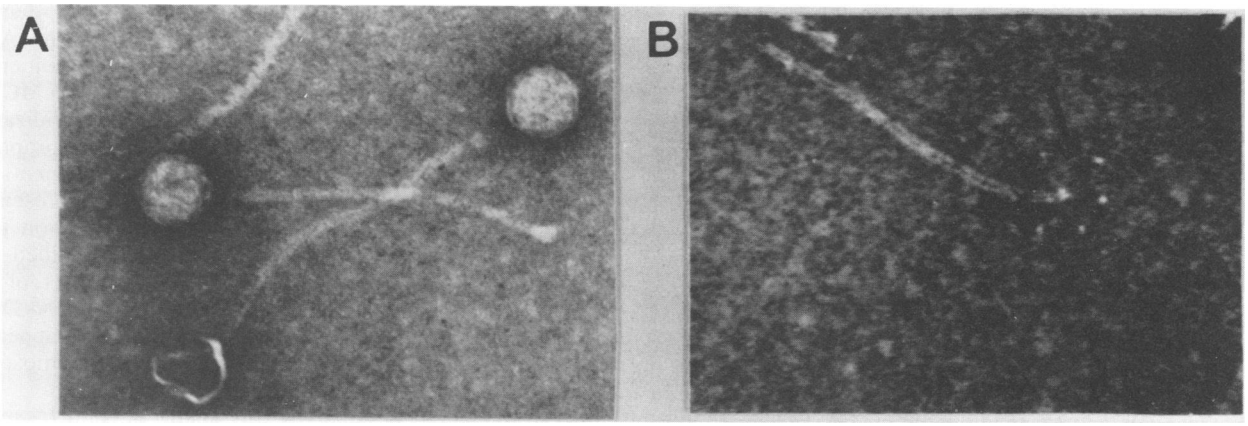

FIG. 2. Electron micrograph of ϕadh particles negatively stained with uranyl acetate. (A) Magnification, ×65,000; (B) arrow indicates position of tail fibers.

harbored an inducible prophage. We sought to isolate a prophage-cured strain of *L. acidophilus* ADH on which the temperate phage ϕadh could be examined for lytic and lysogenic cycles of replication. Initially, ADH cells were plated directly on MRS agar containing 0.02 μg of MC per ml. Survivors were isolated at a frequency of 10^{-2}. Some 300 survivors were further evaluated, but all continued to exhibit lysis upon treatment with MC in broth. *L. acidophilus* ADH cells were also treated with a prophage-inducing dose of UV light, resulting in a 3-log reduction in the initial CFU per milliliter. Twelve clones which survived UV exposure were further evaluated for induction of cell lysis by treatment with MC. Of 12 clones, 6 did not exhibit MC-induced lysis (data not shown).

A lysate induced from *L. acidophilus* ADH, demonstrated above to contain phage particles, was evaluated for biological activity on the six clones that did not show inductive lysis by MC. One of the six clones (designated NCK97) was resistant to any lytic action by ϕadh. For the other five clones, the phage preparation lysed cells in broth cultures and, in spot tests, formed clear zones on indicator cell lawns on MRS agar (pH 6.5) plus 10 mM $CaCl_2$. A representative phage-sensitive clone (designated NCK102) was selected for further study. Figure 4 shows the effects of ϕadh on *L. acidophilus* ADH and NCK102 when phage were added externally to actively growing cultures in MRS broth plus 10 mM $CaCl_2$. The growth of ADH was not affected by the addition of phage, whereas NCK102 lysed in the presence of ϕadh.

NCK102 was used in subsequent experiments to determine optimum conditions for phage activity and titration.

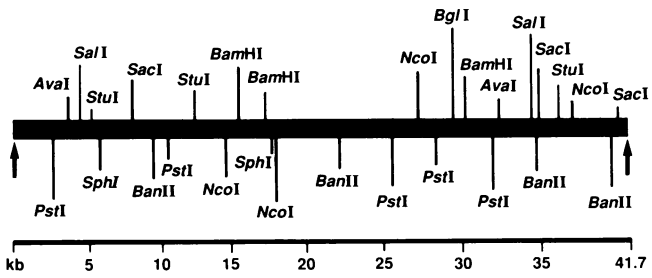

FIG. 3. Restriction enzyme map of the phage ϕadh genome. Map coordinates are in kilobases (kb). Arrows indicate cohesive ends.

Although phage ϕadh induced broth lysis of NCK102 and formed clear zones on agar spot assays, no plaques were detected in standard titration assays on MRS plus $CaCl_2$ agar at pH 6.5. Several different agar media (LBS, MRT, and BHI^+) were evaluated for use in plaque assays. Plaque formation was detected only on LBS agar. Titers as high as 5×10^9 PFU/ml were enumerated. Plaques observed on LBS agar were clear, with a diameter of 1 mm. The major components that distinguish LBS agar medium from the others are the initial low pH (5.5) and the high acetate concentration. To evaluate the effects of these parameters on plaque formation, MRT medium was adjusted with acetic acid to varying initial pHs, and the PFU of ϕadh lysate per milliliter plaqued onto NCK102 were determined. Table 2 shows that pH 5.5 yielded the highest phage titer. Plaquing efficiency was reduced as the pH varied toward 6.1 or 5.2. Values of pH 5.0 or lower were not evaluated due to poor growth of cell lawns. Use of HCl to adjust pH or use of MRS agar at pH 5.5 gave similar results (data not shown). Within the context of the parameters evaluated, these data showed that plaque formation was not related to the type of acid or medium, but rather to the initial agar pH.

Isolation and characterization of NCK103. Strain NCK102

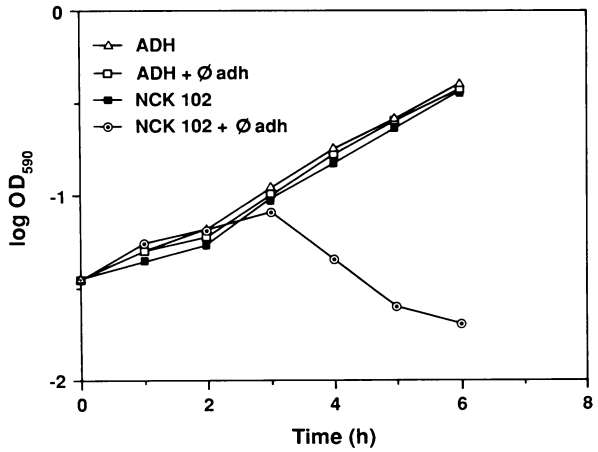

FIG. 4. Effect of infection of ADH and NCK102 cells by ϕadh in MRS broth plus 10 mM $CaCl_2$, pH 5.5. Symbols: △, ADH without ϕadh; □, ADH plus ϕadh; ■, NCK102 without ϕadh; ⊙, NCK102 plus ϕadh.

TABLE 2. Effects of pH on plaque formation by φadh on NCK102

Initial pH[a]	PFU/ml[b]
6.1	<10
5.8	5.0×10^8
5.5	7.0×10^9
5.2	4.1×10^9

[a] pH was adjusted with acetic acid.
[b] Using MRT media.

was infected with phage φadh at a multiplicity of infection of approximately 1. Surviving cells were isolated on LBS agar plus 10 mM $CaCl_2$ and purified. One clone (designated NCK103) was examined for MC induction and sensitivity to φadh. Strain NCK103 was inducible by MC and resistant to plaque formation and lysis by φadh in broth (data not shown).

Lysogeny and superinfection immunity. Demonstration of lytic replication for phage φadh suggested that NCK102 was deficient in superinfection immunity due to curing of prophage φadh. To obtain physical evidence that NCK102 was a prophage-cured derivative of ADH, chromosomal DNAs from several isogenic strains were examined for the presence of φadh DNA sequences. ^{35}S-labeled phage DNA hybridized to *Eco*RI chromosomal DNA fragments from ADH (Fig. 5, lane 4) in a pattern similar to *Eco*RI digests of φadh (Fig. 5, lane 5). In contrast, chromosomal *Eco*RI fragments of NCK102 did not show strong homology with φadh DNA (lane 1); however, bands of weaker hybridization were detected. Common bands were not apparent in comparisons between NCK102 and φadh DNAs. These data demonstrated the presence of the prophage φadh in the ADH chromosome and absence of these phage-specific sequences in NCK102. For NCK97, which did not induce with MC but remained resistant to lytic action by φadh, the hybridization experiment revealed that NCK97 harbored the prophage φadh (Fig. 5, lane 3). Also, hybridization experiments revealed that φadh-specific DNA sequences were present in the NCK103 chromosome (Fig. 5, lane 2). Junction fragments, not detected in *Eco*RI digests, were identified in a subsequent hybridization analysis of *Bcl*I digests of the chromosomal DNA from φadh lysogens (Fig. 5C and D). A 4.3-kilobase *Bcl*I fragment from φadh (lane 6) disappeared and two new bands were visualized in the chromosomal digests of ADH and NCK97 (lanes 7 and 8).

Transduction of plasmid DNA by φadh. Several *L. acidophilus* ADH derivatives harboring antibiotic resistance-marked plasmids (Table 1) were used as a source of transducing phage particles. Transduction of pC194, pGK12, pGB354, and pVA797 to the recipient (NCK101) occurred at frequencies ranging from 3.6×10^{-8} to 8.3×10^{-10} transductants per PFU, using chloramphenicol selection (Table 3). Comparable transducing frequencies were obtained in the presence or absence of 70 μg of DNase I per ml. Transduction of Em^r via the pSA3 and pAMB1 replicons was not detected. Plasmid DNA analysis of representatives from each class of transductant (Table 3) showed that each had acquired intact plasmids and that deletions or rearrangements did not occur as a consequence of transduction. Data for acquisition of plasmid DNA are shown in Fig. 6 for pC194- and pGK12-containing transductants of NCK101.

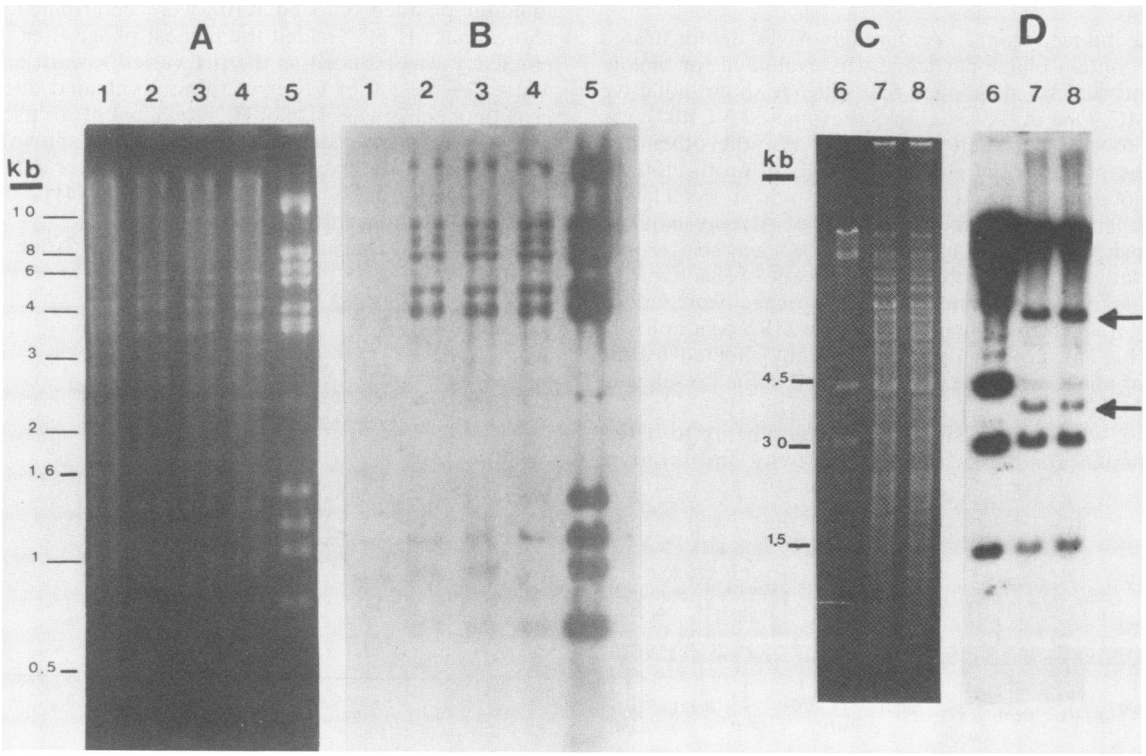

FIG. 5. Demonstration of φadh sequences in *L. acidophilus* lysogens by hybridization. Chromosomal DNA from *L. acidophilus* NCK102 (lane 1), NCK103 (lane 2), NCK97 (lanes 3 and 8), ADH (lanes 4 and 7), and φadh (lanes 5 and 6) DNA. (A and C) Agarose gels of *Eco*RI (A)- and *Bcl*I (C)-digested DNA. (B and D) Autoradiograms obtained after electroblot transfer of DNA to nylon membranes and hybridization with ^{35}S (B)- and ^{32}P (D)-labeled φadh DNA. In panel D, arrows indicate positions of junction fragments. kb, Kilobases.

TABLE 3. Phage ϕadh-mediated transduction of plasmid DNA to *L. acidophilus* ADH (NCK101)

Strain	Plasmid	Selected by:	No. of transductants per:	
			ml	PFU
NCK101	Control	Cm, Em	0	$<1.6 \times 10^{10}$
NCK114	pC194	Cm	86	1.5×10^{8}
NCK111	pGK12	Cm	246	3.6×10^{8}
NCK115	pGB354	Cm	27	1.2×10^{9}
NCK104	pVA797	Cm	7	8.3×10^{10}
NCK117	pSA3	Em	0	$<1.0 \times 10^{10}$
NCK107	pAMB1	Em	0	$<1.0 \times 10^{10}$

Representative transductants of NCK101 were used as donors in second-round transduction experiments: transfer of pGB354 and pGK12 occurred at frequencies of 1.1×10^{-9} and 2.1×10^{-9}, respectively.

Transducing particles were also generated by lytic infection and propagation of phage ϕadh on *L. acidophilus* NCK98(pGK12). The frequencies of pGK12 transduction for lytic phage preparations were similar to those of ϕadh phage induced from NCK111 (data not shown).

DISCUSSION

In this study, the temperate bacteriophage ϕadh was isolated and characterized morphologically, physically, and genetically. Phage ϕadh replicates in a lytic cycle, establishes lysogeny, confers superinfection immunity, and mediates plasmid DNA transduction in *L. acidophilus* ADH. Phage ϕadh belongs to the most common type of phage, those comprising Bradley's group B1 (5) and the *Siphoviridae* family (27). Phage ϕadh is similar to the *L. casei* phages J-1 and PL-1 (1, 42). These phages have a hexagonal head of 60 nm and a long, noncontractile, striated tail. Their genomic DNA is a double-stranded molecule approximating 40 kilobases containing cohesive ends (34, 38). Differences between *L. casei* PL-1 phage and ϕadh were apparent in tail length and tail fibers: PL-1 presents a single short fiber on a tail of 282 nm (38, 42), while phage ϕadh has at least five short fibers on a tail approximating 400 nm. Multiple fibers on the end of a 180-nm tail were detected for the temperate phage 0448 (8) that forms lysogens of *L. bulgaricus* LT4. However, phage 0448 presents a triple collar, while in phage ϕadh this structure is absent. Mata and Ritzenthaler (26) proposed that *L. delbrueckii* subsp. *lactis* phage LL-H and *L. delbrueckii* subsp. *bulgaricus* phage C5 may be used as reference phages for taxonomic studies. Preliminary observations showed that phages ϕadh, LL-H, and C5 are different in host specificity (unpublished data). Whether or not they share DNA homology has not been determined.

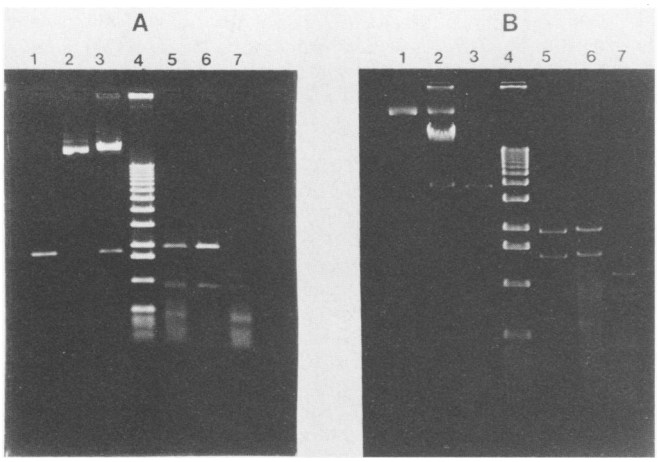

FIG. 6. Plasmid DNA analysis of NCK101 transductants. (A) Lanes 1 plus 6, pC194; lanes 2 plus 7, NCK101 (pTRK15); lanes 3 plus 5, NCK101 transductant (pTRK15 plus pC194). (B) Lanes 1 plus 7, NCK101 (pTRK15); lanes 2 plus 6, NCK101 transductant (pTRK15 plus pGK12); lanes 3 plus 5, pGK12. Lanes 1, 2, and 3 are uncut DNA. Lanes 5, 6, and 7 are *Mbo*I-digested DNA. Lane 4, 1-kilobase ladder.

Phage ϕadh is a temperate phage exhibiting classic lytic and lysogenic cycles of replication. A prophage-cured derivative of strain ADH (NCK102) supported lytic development of ϕadh and served as a phage-sensitive host where lysogeny could be reestablished. When present as a prophage in ADH lysogens, ϕadh conferred immunity to superinfecting phage. Phage ϕadh was also present and expressed superinfection immunity in NCK97, a derivative noninducible by MC. The presence of cohesive ends on ϕadh and detection of junction fragments on the ADH chromosome indicate that prophage integration involves circularization of linear DNA by cohesive end-joining followed by chromosomal integration via a Campbell-like recombination event analogous to phage lambda.

Although lysogeny is widespread among the lactobacilli, few indicator strains have been found for propagation of temperate phages (8, 44). Noting the single exception for the temperate phage from *L. acidophilus* ATCC 19992 (44), in previous reports in which temperate phages were induced from *L. acidophilus*, phagelike particles that were observed in the lysate failed to form plaques or produce progeny phage on *L. acidophilus* indicators. These lysates did, however, form zones of clearing when spotted onto selected *Lactobacillus* cell lawns and were termed "killers" (12). Similar results were reported for six *L. salivarius* strains in which the induced phages could not produce plaques on prophage-cured derivatives (40). In our experiments, although ϕadh produced inhibition zones on a prophage-cured derivative (NCK102) at pH 6.5, plaque formation was not detected. Under these conditions, the spot lysis reactions were identical to 'killer' particle reactions described by de Klerk and Hugo (12). The initial pH of the agar medium used for titration assays was the critical factor that affected the ability and efficiency at which ϕadh produced plaques on NCK102 cell lawns. It was interesting that either organic or inorganic acids were acceptable for adjustment of pH, as long as the initial pH approached 5.5. The role of pH in plaque formation by ϕadh was not investigated. However, these observations encourage close attention to the environmental parameters used in titration assays when attempting to promote lytic activity by *Lactobacillus* phage.

The results presented in this study also demonstrated that phage ϕadh mediates plasmid transduction in *L. acidophilus* ADH. With the exception of pGKV1, the highest frequencies of plasmid transduction were observed for small plasmids (pC194 and pGK12). In electroporation experiments, it was observed previously that pGKV1 was transferred at frequencies of 2 to 3 log cycles lower than pC194, pGK12, and pGB354 (25). One plausible explanation for low-frequency transfer of pGKV1 to *L. acidophilus* may be poor expression of the selectable genetic determinant. Plasmid pGKV1 harbors a *cat* gene from *Bacillus pumilis*, controlled by the phage SPO2 promoter (41), which may be expressed poorly in *L. acidophilus*. In contrast, the *cat* gene of pC194

(present on pGK12; 22) and the *cat* gene of pIP501 (present on pGB354 and pVA797; 4, 15) are expressed efficiently in ADH (25). Similarly, transduction of pSA3 or pAMB1 was not observed. Erythromycin has been a poor marker for direct selection of transformants or transconjugants of *L. acidophilus* ADH (24a, 25); therefore, we believe that, like pGKV1, pSA3- and pAMB1-containing transductants were not selected effectively. Nevertheless, several plasmid cloning vectors of varying size were transferred to ADH cells without apparent deletions or rearrangements. These observations, and the low frequency of transduction in first- and second-round experiments, indicate that nonphage DNA is randomly packaged in a process typical of generalized transducing phages.

The study and characterization of *Lactobacillus* phage should prove extremely useful for application of molecular technologies (genetic mapping and gene transfer) to this economically important group of bacteria. Transduction is an effective means to deliver plasmids and transposons through phage-mediated interspecific and intergeneric genetic transfer systems, as well as to promote characterization of genetically ill-defined bacteria (3, 13, 14, 28–30). In this regard, the ability of phage ϕadh to mediate plasmid transduction is expected to facilitate our development of molecular technologies for lactobacilli as well as to accelerate genetic characterization of *L. acidophilus* ADH.

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