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Purification and Characterization of VSH-1, a Generalized Transducing Bacteriophage of *Serpulina hyodysenteriae*

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Serpulina hyodysenteriae **B204 cells treated with mitomycin (20 μg of mitomycin/ml of culture broth) lysed and released bacteriophages. Bacteriophage particles, precipitated by using polyethylene glycol and purified by CsCl density gradient ultracentrifugation, had a buoyant density of 1.375 g/cm^3 and consisted of a head (45-nm diameter) and an ultrastructurally simple (noncontractile) tail (64 by 9 nm) composed of at least 13 proteins with molecular masses ranging between 13 and 101 kDa. The purified bacteriophage has been designated VSH-1 (VSH for virus of *S. hyodysenteriae*). VSH-1 was incapable of lytic growth on any of five intestinal spirochete strains, representing three *Serpulina* species. VSH-1 nucleic acid was determined to be approximately 7.5 kb in size and to be linear, double-stranded DNA based on differential staining with acridine orange, DNase I sensitivity, electrophoretic mobility, and contour length as measured by electron microscopy. Phage DNA digested by the restriction enzymes *Ssp*I, *Ase*I, *Eco*RV, and *Afl*II gave electrophoretic banding patterns nearly identical to those of digested chromosomal DNA from *S. hyodysenteriae*. Additionally, VSH-1 DNA fragments hybridized with probes complementary to *S. hyodysenteriae* chromosomal genes *nox* and *flaA1*. When purified bacteriophages induced from cultures of *S. hyodysenteriae* A203 (Δ*flaA1* 593-762::*cat*) were added to growing cells of strain A216 (Δ*nox* 438-760::*kan*), transductants (Cm^r Km^r) were obtained at a frequency of 1.5×10^{-6} per phage particle (enumerated by electron microscopy). These findings indicate that induced VSH-1 virions package DNA of *S. hyodysenteriae* and are capable of transferring host genes between cells of that spirochete. To our knowledge, this is the first report of genetic transduction of a spirochete.**

Natural gene transfer mechanisms (conjugative plasmids and transducing bacteriophages) have not been demonstrated for spirochetes. Indirect evidence of lateral gene transfer has been obtained for *Borrelia* spp. (16). The existence of DNA-filled membrane vesicles on the surfaces of *Borrelia burgdorferi* cells has been reported, although their possible role in gene transfer is unknown (7). In addition, several extrachromosomal elements have been identified in *B. burgdorferi*, and an extrachromosomal plasmid of 2.6 kb has been isolated from *Treponema denticola* and characterized (10, 25). In cultures of various spirochetes, bacteriophages have been observed free, attached to cells, or within cells as noted previously (9). Three lytic phages of *Leptospira biflexa* have been isolated and characterized (24). However, to our knowledge, there is no evidence that these three phages play a role in gene transfer.

The spirochete *Serpulina hyodysenteriae* causes swine dysentery, an enteric disease producing a severe mucohemorrhagic diarrhea in infected swine (8). Potential genetic transfer elements for *S. hyodysenteriae* have been identified. Ritchie and colleagues observed bacteriophages with the same morphology in 18 different cultures of *S. hyodysenteriae* while searching for bacteriophages in pure cultures obtained from a collection of cultures from all over the world (22). The extraction of extrachromosomal nucleic acid from *S. hyodysenteriae* cultures was reported in 1986 by Joens and colleagues (11) and then again in 1992 by Combs and coworkers (6). A band of extrachromosomal nucleic acid migrated in agarose gels to a position corresponding to 6 to 8 kb for that of linear double-stranded DNA. Adachi and colleagues detected three plasmids (1.6, 2.6, and 40 kb) in *S. hyodysenteriae*-like spirochetes (2).

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We previously reported an inducible prophage in *S. hyodysenteriae* and *S. innocens* cells (9). In this article we extend those findings by describing the purification and characterization of a mitomycin-inducible phage, designated VSH-1, from *S. hyodysenteriae* B204 cells. VSH-1 bacteriophage packages 7.5-kb fragments of host chromosomal DNA and appears incapable of lytic growth. Furthermore, purified virions were determined to transduce either *flaA1* or *nox* gene between *S. hyodysenteriae* strains, indicating VSH-1 can serve as a generalized transducing phage.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. Cells of *S. hyodysenteriae*, *S. innocens* B256, and *Serpulina pilosicoli* (32) were routinely cultured in BHIS broth beneath a 1% O_2 atmosphere (27). *S. hyodysenteriae* A203, a strain with a mutation in a flagellar core protein gene (Δ*flaA1* 593-762::*cat*), and strain A216, a NADH oxidase (*nox*) mutant (Δ*nox* 438-760::*kan*), were used in transduction experiments. Both strains A203 and A216 were supplied by Everett Rosey and were constructed from *S. hyodysenteriae* B204 (12) by electroporation-mediated allelic exchange (23).

For bacteriophage purification, *S. hyodysenteriae* cells were cultured in NT broth, a low-protein and serum-free medium prepared as follows. Brain heart infusion broth was ultrafiltered (Ultrafiltration cell; 10,000-molecular-weight [MW] cutoff filter YM10; Amicon, Inc., Beverly, Mass.) to remove proteins with high molecular mass and the filtrate used to make basal medium under anaerobic conditions (27). The autoclaved basal medium, 400 ml in a 500-ml sidearm Klett-type flask, was supplemented with 2 ml of an ethanol solution of cholesterol (final concentration in the medium, 25 μg/ml) and phosphatidylcholine (final concentration in the medium, 50 μg/ml). Cultures in NT broth were stirred and incubated under an initial N_2-O_2 (99:1) atmosphere at 37°C. Culture media for strains A203 and A216 contained chloramphenicol (10 μg/ml) and kanamycin (200 μg/ml), respectively.

Induction and purification of VSH-1 particles. Mitomycin was added to cultures of spirochete cells in exponential growth phase (optical density at 620 nm [OD_{620}], 0.9; 8×10^7 cells/ml) in NT broth. A mitomycin concentration (final

concentration, 20 μg/ml) that produced a gradual decrease (0.1 to 0.2 U) in the optical density of the culture within 5 to 8 h after the addition of mitomycin was selected.

VSH-1 was purified by polyethylene glycol (PEG) precipitation and CsCl density gradient ultracentrifugation based on methods for purification of λ phage (26). Culture lysates were treated with DNase I (final concentration, 0.2 μg/ml) and RNase (10 μl/400 ml; RNace-it [Stratagene Inc., La Jolla, Calif.]) for 1 h at 37°C. NaCl (final concentration, 1 M) and $CHCl_3$ (final concentration, 1.5%) were then added, and the lysate was placed on ice. After 1 h, the lysate was centrifuged at 4,000 × *g* for 5 min at 4°C. After a second centrifugation, the supernatant was harvested, and PEG (MW, 8,000) was added (final concentration, 10%). After 48 h at 4°C, the precipitated phage was pelleted by centrifugation (4,000 × *g*, 10 min, 4°C), resuspended in 2 ml of SM buffer (26), and treated with DNase I (final concentration, 8 μg/ml) and RNase (RNace-it, 1 μl/ml) at 37°C for 1 h. The phage suspension was extracted twice with an equal volume of chloroform to remove PEG. Cesium chloride (437 mg/ml) was added to the aqueous phase, and the solution (final density, 1.32 g/cm^3) was ultracentrifuged at 149,000 × *g* for 24 h at 4°C. Phage particles forming a band in the lower half of the ultracentrifuge tube were collected and ultrafiltered (Microcon-100 with a MW cutoff of 100,000; Amicon, Inc.) to remove CsCl. The phage particles were resuspended in SM buffer (0.5 ml), harvested by ultrafiltration twice, and resuspended in 200 μl of SM buffer.

VSH-1 virion characterization. Purified VSH-1 phage was negatively stained by mixing phage samples with an equal volume of phosphotungstic acid (2%, pH 7.0). Samples were deposited on Parlodion-coated 200-mesh carbon-reinforced copper grids and viewed with a Philips model 410 electron microscope (80 kV). Size measurements were made on viruses in electron micrographs from two separate preparations. A total of 100 particles in 20 different fields were measured. VSH-1 bacteriophage was quantified by electron microscopy after phage preparations were mixed (1:1) with dilutions of latex beads (diameter, 0.087 μm; catalog no. 10530 [Ernest Fullam Inc., Latham, N.Y.]) of known concentration. The buoyant density of VSH-1 particles was estimated by measuring the refractive index of samples from purified CsCl phage bands in three preparations by using a refractometer (ERMA Abbe's Refractometer [Fisher, Inc., Pittsburgh, Pa.]).

VSH-1 proteins were characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 3.75% acrylamide stacking gel over a 12% acrylamide resolving gel (0.75 mm thick). A vertical gel electrophoresis apparatus (Mini-Protean II), molecular mass standards, and reagents were purchased from Bio-Rad Laboratories (Hercules, Calif.). Preparation of buffers and staining of proteins with Coomassie blue were done following the manufacturer's recommendations.

Protein concentrations were determined by using Peterson's modification of the Lowry technique (21) or a commercial microprotein assay kit (Bio-Rad). Bovine serum albumin (0.5 mg/ml) (OD_{280} = 0.33 ± .01; 1-cm path length) was used as a standard.

VSH-1 nucleic acid analysis. To extract VSH-1 nucleic acid, purified phage was first treated with DNase I (final concentration, 16 μg/ml) and RNase (RNace-it, 1 μl/ml), for 45 min at 37°C to digest nucleic acid not contained within the virus. Proteinase K (final concentration, 50 μg/ml), SDS (final concentration, 0.5%), and EDTA (final concentration, 20 mM) were added, and the solution was incubated for 15 min at 65°C. The solution was then extracted with an equal volume of phenol-chloroform and then chloroform. A 0.1× volume of sodium acetate (3 M, pH 7.0) and 2× volume of 100% ethanol were added, and the solution was stored at −70°C for 1 h. The precipitated nucleic acid was pelleted by centrifugation (14,000 × *g*, 15 min) in a microcentrifuge.

Various methods were used to determine the nature of VSH-1 nucleic acid. VSH-1 nucleic acid (equivalent to 100 ng of DNA) was treated at 37°C for 1 h with either DNase I (8 μg/ml) or RNase (RNace-it, 1 μl/ml). The nucleic acid was analyzed for degradation by agarose gel electrophoresis (1% agarose gel). To determine whether VSH-1 nucleic acid was single or double stranded, 500-ng preparations of VSH-1 nucleic acid, control double-stranded DNA (pBluescript II SK$^-$; Stratagene), and control single-stranded DNA (M13mp18; United States Biochemical, Inc., Cleveland, Ohio) after agarose electrophoresis (4°C, 3 h, 70 V) were stained with acridine orange (19). Contour length and conformation of VSH-1 nucleic acid were determined by electron microscopy. VSH-1 DNA and circular plasmid DNA (pBluescript II SK$^-$; 2.96 kb) were prepared by the aqueous monolayer spreading technique described by Kleinschmidt (13). Monolayers of nucleic acid and cytochrome *c* were absorbed onto Parlodion-coated 200-mesh grids (carbon reinforced on back sides) and stained with a solution containing uranyl acetate (10 μl of 0.05 M uranyl acetate in 0.05 M HCl mixed with 90% ethanol). The grids were then coated with platinum-palladium while rotating in a vacuum shadower and examined with an electron microscope. A map measurer was used to obtain measurements of individual molecules. The average sizes of 20 molecules each of VSH-1 DNA and of pBluescript II SK$^-$ DNA were determined.

Restriction enzyme digestion and Southern hybridization analyses of VSH-1 nucleic acid. Genomic DNA was isolated from *S. hyodysenteriae* B204 cells in the exponential phase of growth (29). VSH-1 DNA (2 μg) and genomic DNA (2 μg) were digested overnight with various restriction enzymes following the manufacturer's instructions. The resulting DNA fragments were separated by electrophoresis on a 1% agarose gel (100 V; 3 h) using 0.5× TBE buffer (26), stained with ethidium bromide (0.5 μg/ml), transilluminated with UV light, and photographed. A downward blotting technique (5) was used to transfer the DNA fragments onto nylon membranes (Hybond N; Amersham, Arlington Heights, Ill.). The DNA was then cross-linked to the membrane with UV light (Stratagene 2400 UV cross-linker; Stratagene) for use in Southern hybridization experiments.

Southern hybridizations used either a *nox* gene probe (5′-ATGAAAGTTAT TGAATAGG-3′) corresponding to base positions 1 to 20 in the *nox* coding region (28) or a probe (5′-GCGGTGCTGATGGTACTAA-3′) corresponding to base positions 367 to 385 in the *flaA1* gene (14). The probes were labeled at the 5′ end with [γ-^{32}P]dATP (26). Prehybridization and hybridization buffers consisted of the following (final concentrations given): 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (26), 5× Denhardt's solution, 0.1% SDS, and 200 μg of denatured salmon sperm DNA per ml. Prehybridization was for 2 h at 50°C, and hybridization was overnight at 50°C with 4 μl of radiolabeled probe (15 pmol total; approximately 8 × 10^4 cpm) per ml in 10 ml of hybridization solution. Hybridization was followed by three washes at room temperature for 1 min each and a final wash at 50°C for 30 min. Wash buffer consisted of 5× SSC containing 0.5% SDS. Kodak X-Omat AR scientific imaging film was used for autoradiography.

Assays for VSH-1 lytic growth. *S. hyodysenteriae* B204 and B78, *S. pilosicoli* P43/6/78 and Wes-B, and *S. innocens* B256 were tested as hosts for VSH-1 lytic growth. Approximately 10^8 cells of each strain were uniformly streaked with an inoculation loop across a Trypticase soy agar plate supplemented with 5% bovine blood (TSB). Purified VSH-1 phage particles (10^{11}) were spotted (3 μl) in the center of the streak, and the plates were incubated at 39°C in an anaerobic chamber. After 5 days, the plates were examined to detect VSH-1 inhibition of bacterial growth.

Genetic transduction analysis. To determine whether or not bacteriophage VSH-1 is a generalized transducing phage, VSH-1 particles were induced from *S. hyodysenteriae* A203 (Δ*flaA1* 593-762::*cat*). During purification, these VSH-1 particles were treated three times with DNase I to digest any DNA not protected by VSH-1 proteins (i.e., not contained within virions). These virions were then added to *S. hyodysenteriae* A216 (Δ*nox* 438-760::*kan*) cells in the early exponential phase of growth (OD_{620} = 0.9; 8 × 10^7 cells/ml) in tubes of BHIS broth containing kanamycin (200 μg/ml). A total of 8.2 × 10^{10} VSH-1 particles (46 μg of protein; virion/cell ratio, 150:1) were added to 7-ml cultures. Culture samples were taken hourly and plated (200 μl) onto TSB plates and incubated at 39°C in an anaerobic chamber. TSB agar containing chloramphenicol (10 μg/ml) and kanamycin (200 μg/ml) was used to screen for transductants.

Southern blot analysis was performed on transductants in order to detect the chloramphenicol resistance gene and confirm that homologous recombination between the VSH-1 packaged *flaA1*::*cat* gene construct and the recipient's wild-type *flaA1* gene had taken place. DNAs isolated from three randomly chosen transductants were digested with restriction endonucleases and analyzed by Southern hybridization to the *flaA1* gene probe as described above.

Additionally, to confirm that transductants contained the *flaA1*::*cat* gene construct, PCRs were performed with two primer sets specific for this locus. The first set contained a primer specific for the 5′ end of the *flaA1* gene and a primer specific for the 5′ end of the inserted chloramphenicol resistance gene. The second set of primers consisted of specific primers complementary to regions of the *flaA1* gene which flank the chloramphenicol resistance gene. The *flaA1* 5′-end primer ERL10 (5′-GGGGATCCTATGAAAAAGTTATTCGTAGTAT TAACTTTCC-3′, positions 1 to 31 [23]) and the *cat* 5′-end primer ERL16 (5′-GATTAAAGATCTCTTTTCTCTTCC-3′, positions 55 to 32 [23]) were used for the first primer set. *flaA1* primers 601 (5′-GCGGTGCTGATGGTACTAA-3′, positions 367 to 385 [14]) and 602 (5′-TAGCAGCAGCACCTTGATC-3′, positions 915 to 897 [14]) were used for the second primer set. Amplification of DNA was carried out using 100 ng of purified genomic DNA as a target. Amplitaq DNA polymerase, ampliwax gems, and PCR core reagents (Roche Molecular Systems, Inc., Branchburg, N.J.) were used in PCR DNA amplification according to the manufacturer's recommendations. Samples were amplified in a thermocycler (Uno; Biometra Inc., Tampa, Fla.) for 36 cycles, with each cycle consisting of denaturation (95°C, 1 min), annealing (48°C, 1 min), and extension (72°C, 2 min) steps, followed by a final extension step at 72°C for 8 min. PCR products were separated by agarose gel electrophoresis, stained with ethidium bromide, and detected by UV transillumination.

RESULTS

VSH-1 purification and virion properties. Previously, we described the induction of bacteriophage from *S. hyodysenteriae* B204 in BHIS broth to which mitomycin was added (final concentration, 8 μg/ml) (9). In the present study, two factors were found to be important for successful purification of the *S. hyodysenteriae* bacteriophage we have designated VSH-1. Bacterial cells had to be cultured in NT broth to avoid precipitating large amounts of culture medium protein during purification of the virions and mitomycin concentrations had to be

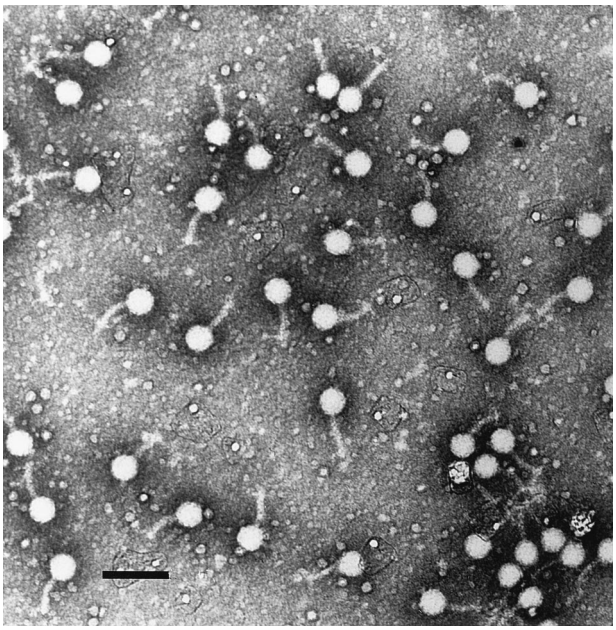

FIG. 1. Electron micrograph of VSH-1 virions. Purified VSH-1 particles from mitomycin-treated cultures of *S. hyodysenteriae* B204. Virions were negatively stained with 2% phosphotungstic acid, pH 7.0. Bar = 0.1 μm.

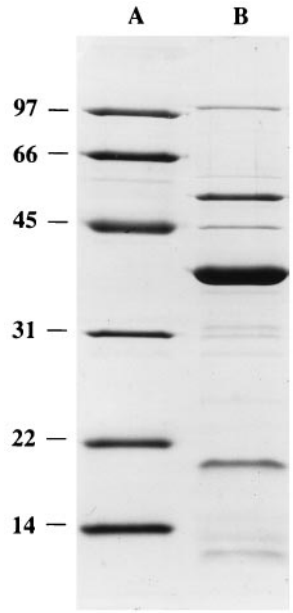

FIG. 2. SDS-PAGE of purified VSH-1 virions. Denatured bacteriophage proteins were separated by electrophoresis (12% acrylamide gel, 175 V, 1 h, 25°C). Lanes: A, low-molecular-size markers (Bio-Rad; 6.5 μg) (molecular masses [in kilodaltons] indicated to the left of the gel); B, VSH-1 phage preparation (4 μg).

raised to 20 μg/ml (NT broth) for effective induction of the phage.

After ultracentrifugation of PEG-precipitated virus, phage particles formed a visible band three-fourths of the distance from the top of the CsCl gradient. Based on the refractive index measurements of the CsCl solution at the position of the phage band, the VSH-1 virions had a buoyant density of 1.375 g/cm^3. Additional bands near the top of the CsCl gradient consisted primarily of flagella, tailless heads, and clumps of VSH-1 virions as determined by electron microscopy.

VSH-1 particles (Fig. 1) were of uniform size and consisted of a head (45-nm diameter) and a ultrastructurally simple (noncontractile) tail (64 by 9 nm). Analysis of purified phage preparations by SDS-PAGE (Fig. 2) revealed 13 Coomassie blue-staining protein bands corresponding to proteins with a range of molecular masses between 13 and 101 kDa. Six of these bands (101, 53, 45, 38, 19, and 13 kDa) stained intensely. The 38-kDa band was prominent (Fig. 2), suggesting that this protein is the most abundant and likely to be the major head protein of VSH-1.

Tests for lytic growth. Five different *Serpulina* strains did not support detectable lytic growth of VSH-1. Therefore, it is not likely that VSH-1 grows on or lyses cells of *S. hyodysenteriae* B204 and B78, *S. pilosicoli* P43/6/78 and Wes-B, or *S. innocens* B256. In addition, we have not detected spontaneous production (no mitomycin added) of VSH-1 from *S. hyodysenteriae* B204 cells under various culture conditions (unpublished observations).

VSH-1 nucleic acid. Before extraction from purified virions, VSH-1 nucleic acid was resistant to DNase I treatment, but it became sensitive after extraction. Extracted nucleic acid was resistant to RNase treatment. These results indicated that the nucleic acid packaged inside VSH-1 particles was DNA. VSH-1 nucleic acid in electrophoretic gels fluoresced green under UV light when stained with acridine orange. Control DNAs, namely, M13mp18 and pBluescript II SK$^-$, fluoresced orange and green, respectively, under the same conditions. These results and the ability of the VSH-1 DNA to be digested by restriction enzymes (see below) indicated that VSH-1 DNA was double stranded. Electron microscopy observations of VSH-1 nucleic acid revealed a linear molecule (Fig. 3) whose contour length corresponded to a 7.5-kb molecule of DNA. This size is similar to size estimates based on electrophoretic migration of untreated phage DNA (Fig. 4A). Thus, VSH-1 virions contain 7.5-kb, double-stranded, linear DNA molecules.

When restriction enzyme digestion patterns for VSH-1 DNA and *S. hyodysenteriae* B204 chromosomal DNA were compared, the banding patterns of viral and bacterial DNA were virtually identical to each other in the fragment size range of 0.6 to 6 kb (Fig. 4A). For the *Eco*RV and *Afl*II digestions of VSH-1 DNA, no DNA fragments were observed above 7.5 kb, the estimated size of untreated VSH-1 DNA (Fig. 4A, lane B). This finding suggested that VSH-1 contained *S. hyodysenteriae* chromosomal DNA.

Restriction fragments of VSH-1 DNA and *S. hyodysenteriae* B204 DNA were analyzed by Southern blot techniques (Fig. 4B). A NADH oxidase gene probe (*nox*) gave a positive reaction for both viral and bacterial DNA (Fig. 4B). The *nox* probe hybridized to DNA fragments of identical size (approximately 1.4 kb) for both VSH-1 and B204 in the *Ssp*I and *Hin*fI digest patterns (Fig. 4B, lanes D to G). The *nox* probe hybridized to a B204 DNA fragment of 7.5 kb in the *Eco*RV digest and a DNA fragment greater than 10 kb in the *Afl*II digest, whereas a weak and diffuse hybridization reaction was obtained from VSH-1 DNA (*Eco*RV and *Afl*II restriction patterns) around the size range of 6 to 7.5 kb. When the DNA fragments were hybridized with a *flaA1* gene probe, similar results were obtained (unpublished observation). These results indicate that VSH-1 contains bacterial genes and that the induced virus packages host DNA in randomly generated 7.5-kb fragments.

Genetic transduction. When purified VSH-1 particles induced from *S. hyodysenteriae* A203 (*flaA1*::*cat*) cells were

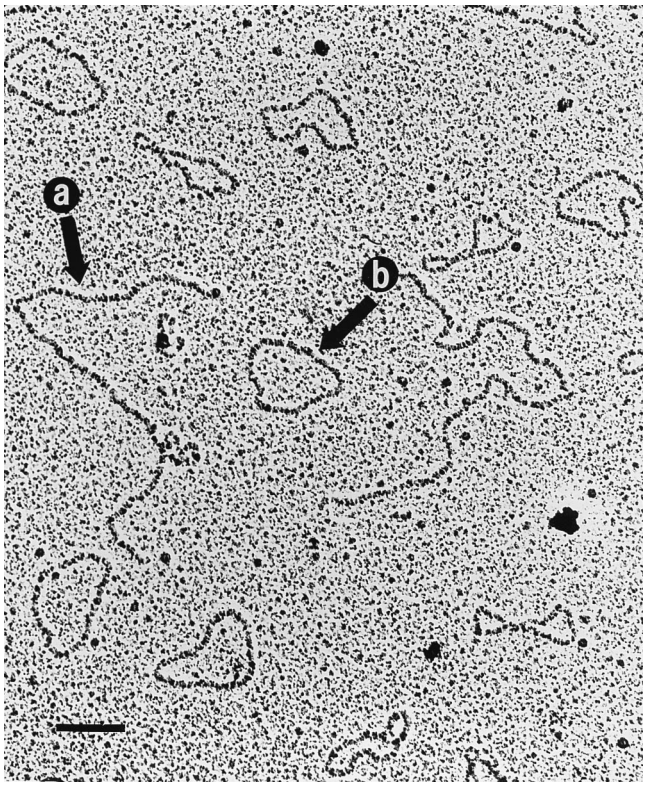

FIG. 3. Electron micrograph of VSH-1 DNA. Linear double-stranded VSH-1 DNA (a) mixed with circular plasmid pBluescript II SK$^-$ (2.96 kb) (b). Marker bar = 0.2 μm.

added to growing cells of *S. hyodysenteriae* A216 (*nox*::*kan*), cells that were resistant to both chloramphenicol and kanamycin were isolated from the cultures at a maximum frequency of 1.5×10^{-6} CFU per phage particle after 8 h of incubation of virus with spirochete cells. No doubly resistant cells were recovered from control cultures (virus not added) in any experiment.

To confirm that the transductants contained the transferred *flaA1*::*cat* gene construct, DNAs isolated from three randomly selected transductants (strains SH, JH, and BH) were analyzed by Southern blotting using the *flaA1* gene probe (Fig. 5). DNA hybridization fragment patterns of transductants were the same as those of A203 (Fig. 5), indicating homologous recombination had occurred. In separate experiments, when the NADH oxidase gene was probed with the *nox* gene probe, results indicated that the transductants had retained the *nox*::*kan* gene construct present in the original recipient (data not shown).

For additional confirmation that the transductants contained the *flaA1*::*cat* gene construct, PCR was performed on DNA extracted from transductants. PCR amplification of the *flaA1* gene of the transductants with primers from *flaA1* and the chloramphenicol resistance gene yielded products whose sizes indicated the insertion of the *flaA1*::*cat* gene construct at the *flaA1* gene locus. The existence of the *nox*::*kan* gene construct in the transductants was also confirmed by a similar approach.

From these transduction experiments, we conclude that chromosomal DNA from strain A203 was packaged into induced VSH-1, the purified particles then transferred this DNA into strain A216, and recombination at the *flaA1* locus took place. In other experiments, reverse transduction was attempted. VSH-1 virions were purified from strain A216 (*nox*::*kan*) and added to cultures of strain A203 (*flaA1*::*cat*). Transductants were obtained at the same frequency as in the experiment described above. It should also be noted that gene transfer between strains occurred when mitomycin-treated cultures and untreated cultures were mixed.

In preliminary experiments (unpublished observations), maximum transduction frequencies were obtained 8 h after bacteriophage VSH-1 was added to *S. hyodysenteriae* cultures. Increasing the ratio of bacteria to bacteriophage increased the efficiency of transduction. Adding $MgSO_4$ (50 mM) or $CaCl_2$ (10 mM) to the BHIS growth medium had no detectable effect on transduction frequency.

DISCUSSION

Bacteriophage particles have been observed attached to cells of *S. hyodysenteriae* in cultures (22), and the use of mitomycin to induce bacteriophage from the spirochete *S. hyodysenteriae* has been described previously (9). The experiments in this article describe the isolation and characterization of a bacteriophage, designated VSH-1, from *S. hyodysenteriae* B204 cells after treatment with mitomycin. The morphology and size of purified VSH-1 virions are identical to those of *S. hyodysenteriae* phages described in previous studies (9, 22). VSH-1 virions have a buoyant density in CsCl of 1.375 g/cm^3 and are composed of a head (45-nm diameter) and a simple tail (64 by 9 nm) made from at least 13 structural proteins with molecular masses between 13 and 101 kDa. The morphology of VSH-1 places it in the *Siphoviridae* family of tailed phages, a group that includes phages like coliphage λ, *Bacillus* phage α, and *Staphylococcus* phage 3A (1). When induced with mitomycin, the VSH-1 bacteriophage packages 7.5-kb linear double-stranded DNA fragments. Restriction digest comparisons of VSH-1 packaged DNA and *S. hyodysenteriae* B204 chromosomal DNA revealed identical restriction fragment patterns below 7.5 kb, indicating that VSH-1 packages host chromosomal DNA. No prominent DNA fragments were observed, suggesting no biased packaging and no phage genes detectable due to higher copy number.

Previous investigators have described extrachromosomal DNA approximately 7.5 kb in size in DNA preparations from *S. hyodysenteriae* cells (6, 11). This extrachromosomal DNA can likely be interpreted as VSH-1 virions produced spontaneously (without mitomycin induction), although we have not detected spontaneously produced phage in *S. hyodysenteriae* cultures.

VSH-1 seems incapable of lytic growth based on our inability to detect growth inhibition when purified virions are spotted onto growing cells of three *Serpulina* species. There are several reasons why VSH-1 may be or appears defective for lytic growth. First, the size of the DNA packaged within VSH-1 phage heads (7.5 kb) appears too small to code for all proteins necessary for VSH-1 replication. However, it is possible that VSH-1 packages more than one 7.5-kb fragment of DNA per virion, or phage genes may have overlapping coding regions. Second, VSH-1 may have a packaging defect that renders it nonlytic. Third, *Serpulina* strains tested may harbor prophage with repressors to VSH-1 replication or lack VSH-1 receptors on their cell surfaces. Regarding this point, *S. hyodysenteriae* B78 contains VSH-1-like prophage inducible by mitomycin treatment (9). Finally, lytic growth may not have been detected because the DNA which VSH-1 delivers to *Serpulina* cells would be mostly from the *S. hyodysenteriae* B204 bacterial host.

The packaging of fragments of host chromosomal DNA by a

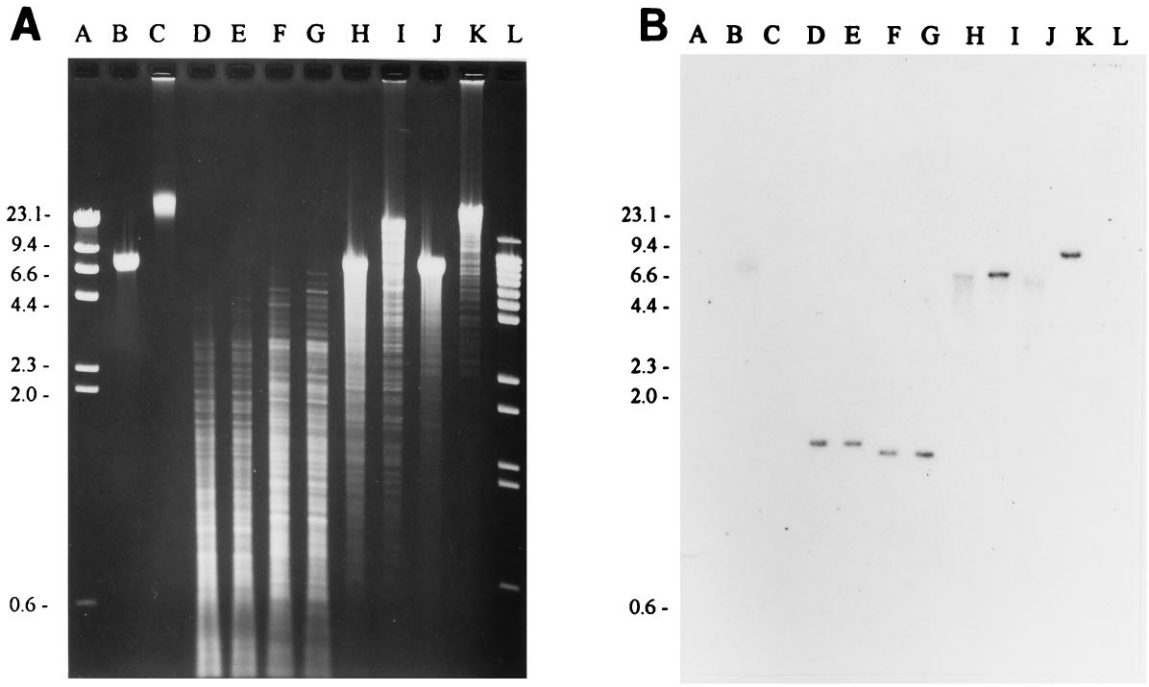

FIG. 4. Comparison of VSH-1 DNA and *S. hyodysenteriae* B204 chromosomal DNA by restriction endonuclease and Southern blot analysis. (A) Electrophoretic separation of VSH-1 and *S. hyodysenteriae* DNA fragments. Lanes: A, *Hin*dIII-digested λ DNA; B, VSH-1 DNA (uncut); C, B204 DNA (uncut); D, *Ssp*I-digested VSH-1 DNA; E, *Ssp*I-digested B204 DNA; F, *Hin*fI-digested VSH-1 DNA; G, *Hin*fI-digested B204 DNA; H, *Eco*RV-digested VSH-1 DNA; I, *Eco*RV-digested B204 DNA; J, *Afl*II-digested VSH-1 DNA; K, *Afl*II-digested B204 DNA; L, *Bst*EII-digested λ DNA. (B) Southern blot analysis of DNA from the gel in panel A. DNA fragments were hybridized with ^{32}P-labeled probe complementary to the *S. hyodysenteriae* NADH oxidase (*nox*) gene. The positions (in kilobases) of DNA fragment size markers are indicated to the left of the gels.

bacteriophage is unusual but not unique. When *Bacillus subtilis* 168 cells are treated with mitomycin, the PBSX bacteriophage is induced and packages 13-kb fragments of host chromosomal DNA (3, 20). Another inducible bacteriophage, PBND8, from a strain of *Bacillus natto* packages host chromosomal DNA in 8-kb fragments (33). In addition, many PBSX-like bacteriophages have been induced with mitomycin from strains of *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus pumilus*, and *Bacillus subtilis* (30). These *Bacillus* phages are termed defective phages. They are not capable of lytic infections or lysogeny and possess bacteriocin-like killing activities when mixed with susceptible strains (15, 20, 30). In our experiments, no evidence was obtained which would indicate VSH-1 possesses bacteriocin-like killing activity. PBSX virions are believed to be defective in packaging, since the DNA content of PBSX virions is too little to code for all of the proteins necessary for phage production (18). PBSX packaging of host chromosomal DNA appears to be somewhat preferential for certain regions of the chromosome, whereas host DNA is packaged nonpreferentially by phage PBND8 (3, 33). Based on restriction digest patterns for VSH-1 DNA, packaging by VSH-1 virions is nonpreferential. PBSX virions are not capable of injecting the DNA they package into *Bacillus* cells (20), and therefore, these virions are not generalized transducing particles. However, from our experiments we have found that VSH-1 not only packages host chromosomal DNA but also is capable of generalized transduction.

The frequency at which VSH-1 transduced either the *flaA1*::*cat* gene construct or the *nox*::*kan* gene construct was 1.5×10^{-6} transductants per phage particle. These markers are located on the opposite side of the *S. hyodysenteriae* chromosome (35), so there does not appear to be preferences in regards to chromosomal location for genetic transduction. Since VSH-1 does not form plaques, the transduction frequency estimates were based on the number of phage particles estimated from direct electron microscope counts. The frequencies of gene transfer by the generalized transducing phage P1 vary from 3×10^{-4} to 10^{-5} per infective phage particle, depending upon the marker being transduced (17). For phage P22, frequencies vary from 10^{-6} to 10^{-9} transductants per infectious particle (34). Thus, VSH-1 transducing frequencies appear comparable to those of other generalized transducing phages. However, it should be pointed out that unlike the other phages that package bacterial DNA in a small fraction of the total number of phage particles produced during lysis, VSH-1 appears to package bacterial DNA in the majority of particles produced after induction. This is the first report of genetic transduction of *S. hyodysenteriae* and, to our knowledge, for any spirochete (order *Spirochetales*).

Transferring genes into *S. hyodysenteriae* cells by electroporation to form stable recombinants has been accomplished (23, 31). From these experimental results, 500 ng of total DNA, consisting of plasmid and construct DNA (*S. hyodysenteriae* gene disrupted with an antibiotic resistance gene), was electroporated into 10^{10} cells to obtain five recombinants. In our experiments, approximately 640 ng of DNA, packaged inside VSH-1, was mixed with 5.6×10^8 cells and a total of 1.7×10^4 transductants were obtained. Taking into account that the electroporation experiments used a single cloned gene and transduction experiments used a heterogeneous population of bacterial DNA fragments, transduction appears to be at least 10^6 times more efficient than electroporation at moving DNA into cells to form stable recombinants. This analysis underscores

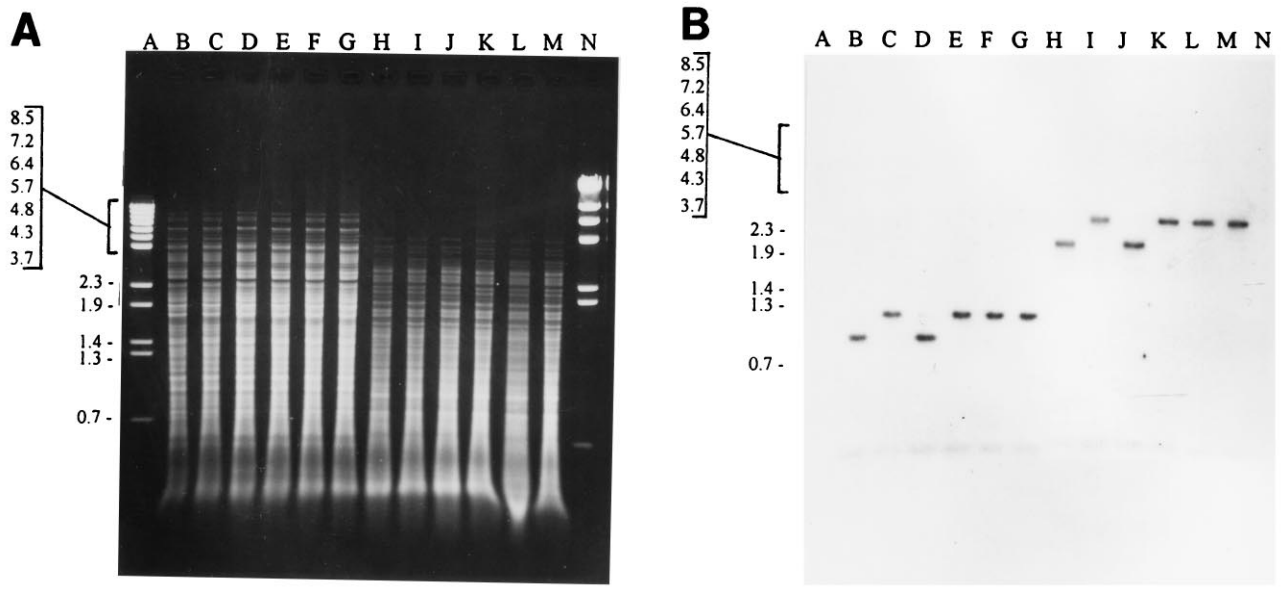

FIG. 5. Analysis of transductant strains DNA by restriction endonuclease and Southern blot analysis. (A) DNA restriction enzyme fragments separated by agarose gel electrophoresis. Lanes: A, *Bst*EII-digested λ DNA; B, *Ase*I-digested B204 DNA; C, *Ase*I-digested A203 DNA; D, *Ase*I-digested A216 DNA; E, *Ase*I-digested isolate SH DNA; F, *Ase*I-digested isolate JH DNA; G, *Ase*I-digested isolate BH DNA; H, *Ssp*I-digested B204 DNA; I, *Ssp*I-digested A203 DNA; J, *Ssp*I-digested A216 DNA; K, *Ssp*I-digested isolate SH DNA; L, *Ssp*I-digested isolate JH DNA; M, *Ssp*I-digested isolate BH DNA; N, *Hin*dIII-digested λ DNA. (B) Southern hybridization of a membrane prepared from the gel in panel A. DNA fragments were hybridized to a ^{32}P-labeled *flaA1* gene probe. DNA was extracted from cells of *S. hyodysenteriae* B204 (wild type), A203 (*flaA1*::*cat*) (source of virus VSH-1), and A216 (*nox*::*kan*) (strain to which purified virus was added) strains and potential transductant isolates SH, JH, and BH.

the need for improved techniques for genetically modifying spirochetes.

The potential for VSH-1 to be utilized as a gene transfer system needs further analysis. Identification of the prophage genes, especially the origin of replication, may lead to the isolation of autonomously replicating DNA molecules, as was accomplished with the *Bacillus* phage PBSX (4). This advance could lead to the development of shuttle vectors useful in studying the genetics of *S. hyodysenteriae* and related spirochetes. Additionally, it is possible that VSH-1 plays an important role in natural gene transfer among populations of *S. hyodysenteriae* cells. The conditions necessary for spontaneous induction of VSH-1 particles from *S. hyodysenteriae* cells need to be determined, and methods for the detection and enumeration of VSH-1 virions (other than electron microscopy) need to be improved. Perhaps at that point it will be possible to understand the significance of VSH-1 to the ecology of *S. hyodysenteriae.*

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REFERENCES

1. **Ackermann, H. W., and M. S. Dubow.** 1995. Tailed phages, p. 49–63. *In* F. A. Murphy, C. M. Fauquet, D. H. Bishop, S. A. Ghabrial, A. W. Jarvis, G. P. Martelli, M. A. Mayo, and M. D. Summers (ed.), Virus taxonomy: classification and nomenclature of viruses. Springer-Verlag Wein, New York, N.Y.
2. **Adachi, Y., M. Hara, and K. Hirano.** 1994. Biological properties of *Serpulina hyodysenteriae*- and *Serpulina innocens*-like organisms with plasmid DNAs, p. 147. *In* P. Poomvises and P. Ingkaninun (ed.), Proceedings of the 13th International Pig Veterinary Society Congress, Chulalongkorn University, Bangkok, Thailand.
3. **Anderson, L. M., and K. F. Bott.** 1985. DNA packaging by the *Bacillus subtilis* defective bacteriophage PBSX. J. Virol. **54:**773–780.
4. **Anderson, L. M., H. E. Ruley, and K. F. Bott.** 1982. Isolation of an autonomously replicating DNA fragment from the region of defective bacteriophage PBSX of *Bacillus subtilis*. J. Bacteriol. **150:**1280–1286.
5. **Chomczynski, P.** 1992. One-hour downward alkaline capillary transfer for blotting of DNA and RNA. Anal. Biochem. **201:**134–139.
6. **Combs, B. G., D. J. Hampson, and S. J. Harders.** 1992. Typing of Australian isolates of *Treponema hyodysenteriae* by serology and by DNA restriction endonuclease analysis. Vet. Microbiol. **31:**273–285.
7. **Garon, C. F., L. L. Lubke, and M. D. Corwin.** 1991. Use of chloro (2,2′:6,2″-terpyridine) platinum (II) chloride as an effective label for nucleic acid containing cellular structures. Scanning **13:**81–82.
8. **Harris, D. L., and R. J. Lysons.** 1992. Swine dysentery, p. 599–616. *In* A. D. Leman, B. E. Straw, W. L. Mengeling, R. H. C. Penny, and E. Scholl (ed.), Diseases of swine, 7th ed. Iowa State University Press, Ames.
9. **Humphrey, S. B., T. B. Stanton, and N. S. Jensen.** 1995. Mitomycin C induction of bacteriophages from *Serpulina hyodysenteriae* and *Serpulina innocens.* FEMS Microbiol. Lett. **134:**97–101.
10. **Ivic, A., J. Macdougall, R. R. B. Russell, and C. W. Penn.** 1991. Isolation and characterization of a plasmid from *Treponema denticola*. FEMS Microbiol. Lett. **78:**189–194.
11. **Joens, L. A., A. B. Margolin, and M. J. Hewlett.** 1986. The characterization of a plasmid isolated from *Treponema hyodysenteriae* and *Treponema innocens*, abstr. H-173, p. 156. *In* Abstracts of the Annual Meeting of the American Society for Microbiology 1986. American Society for Microbiology, Washington, D.C.
12. **Kinyon, M. J., D. L. Harris, and R. D. Glock.** 1977. Enteropathogenicity of various isolates of *Treponema hyodysenteriae*. Infect. Immun. **15:**638–646.
13. **Kleinschmidt, A. K.** 1968. Monolayer techniques in electron microscopy of nucleic acid molecules. Methods Enzymol. **12:**361–377.
14. **Koopman, M. B., O. S. DeLeeuw, B. A. van der Zeijst, and J. G. Kusters.** 1992. Cloning and DNA sequence analysis of a *Serpulina* (*Treponema*) *hyodysenteriae* gene encoding a periplasmic flagellar sheath protein. Infect. Immun. **60:**2920–2925.
15. **Longchamp, P. F., C. Mauel, and D. Karamata.** 1994. Lytic enzymes associated with defective prophages of *Bacillus subtilis*: sequencing and characterization of the region comprising the N-acetylmuramoyl-L-alanine amidase gene of prophage PBSX. Microbiology **140:**1855–1867.
16. **Marconi, R. T., D. S. Samuels, R. K. Landry, and C. F. Garon.** 1994. Analysis of the distribution and molecular heterogeneity of the *ospD* gene among the Lyme disease spirochetes: evidence for lateral gene transfer. J. Bacteriol. **176:**4572–4582.
17. **Masters, M.** 1977. The frequency of P1 transduction of the genes of *Escherichia coli* as a function of chromosomal position: preferential transduction of the origin of replication. Mol. Gen. Genet. **155:**197–202.
18. **Mauel, C., and D. Karamata.** 1984. Characterization of proteins induced by

mitomycin C treatment of *Bacillus subtilis*. J. Virol. **49:**806–812.
19. **McMaster, G. K., and G. C. Carmichael.** 1977. Analysis of single- or double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc. Natl. Acad. Sci. USA **74:**4835–4838.
20. **Okamoto, K., J. A. Mudd, J. Managan, W. M. Huang, T. V. Subbaiah, and J. Marmur.** 1968. Properties of the defective phage of *Bacillus subtilis*. J. Mol. Biol. **34:**413–428.
21. **Peterson, G. L.** 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. **83:**346–356.
22. **Ritchie, A. E., I. M. Robinson, L. A. Joens, and J. M. Kinyon.** 1978. A bacteriophage for *Treponema hyodysenteriae*. Vet. Rec. **103:**34–35.
23. **Rosey, E. L., M. J. Kennedy, D. K. Petrella, R. G. Ulrich, and R. J. Yancey, Jr.** 1995. Inactivation of *Serpulina hyodysenteriae flaA1* and *flaB1* periplasmic flagellar genes by electroporation-mediated allelic exchange. J. Bacteriol. **177:**5959–5970.
24. **Saint Girons, I., D. Margarita, P. Amouriaux, and G. Baranton.** 1990. First isolation of bacteriophages for a spirochete: potential genetic tools for *Leptospira*. Res. Microbiol. **141:**1131–1138.
25. **Saint Girons, I., S. J. Norris, U. Gobel, J. Meyer, E. M. Walker, and R. Zuerner.** 1992. Genome structure of spirochetes. Res. Microbiol. **143:**615–621.
26. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Stanton, T. B., and C. P. Cornell.** 1987. Erythrocytes as a source of essential lipids for *Treponema hyodysenteriae*. Infect. Immun. **55:**304–308.
28. **Stanton, T. B., B. L. Hanzelka, and N. S. Jensen.** 1995. Survey of intestinal spirochaetes for NADH oxidase by gene probe and by enzyme assay. Microb. Ecol. Health Dis. **8:**93–100.
29. **Stanton, T. B., N. S. Jensen, T. A. Casey, L. A. Tordoff, F. E. Dewhirst, and B. J. Paster.** 1991. Reclassification of *Treponema hyodysenteriae* and *Treponema innocens* in a new genus, *Serpula* gen. nov. as *Serpula hyodysenteriae* comb. nov. and *Serpula innocens* comb. nov. Int. J. Syst. Bacteriol. **41:**50–58.
30. **Steensma, H. Y., L. A. Robertson, and J. D. Van Elsas.** 1978. The occurrence and taxonomic value of PBSX-like defective phages in the genus *Bacillus*. Antonie Leeuwenhoek J. Microbiol. **44:**353–366.
31. **ter Huurne, A. A. H. M., M. van Houten, S. Muir, J. G. Kusters, B. A. M. van der Zeijst, and W. Gaastra.** 1992. Inactivation of a *Serpula* (*Treponema*) *hyodysenteriae* hemolysin gene by homologous recombination: importance of this hemolysin in pathogenesis of *S. hyodysenteriae* in mice. FEMS Microbiol. Lett. **92:**109–114.
32. **Trott, D. J., T. B. Stanton, N. S. Jensen, G. E. Duhamel, J. L. Johnson, and D. J. Hampson.** 1996. *Serpulina pilosicoli* sp. nov., the agent of porcine intestinal spirochetosis. Int. J. Syst. Bacteriol. **46:**206–215.
33. **Tsutsumi, Y., H. Hirokawa, and K. Shishido.** 1990. A new defective phage containing a randomly selected 8 kilobase-pairs fragment of host chromosomal DNA inducible in a strain of *Bacillus natto*. FEMS Microbiol. Lett. **72:**41–46.
34. **Yarmolinsky, M. B., and N. Sternberg.** 1988. Bacteriophage P1, p. 291–438. *In* R. Calender (ed.), The bacteriophages, vol. 1. Plenum Press, New York, N.Y.
35. **Zuerner, R. L., and T. B. Stanton.** 1994. Physical and genetic map of the *Serpulina hyodysenteriae* B78^T chromosome. J. Bacteriol. **176:**1087–1092.