INFECTION AND IMMUNITY, July 1985, p. 1–6
0019-9567/85/070001-06$02.00/0

Vol. 49, No. 1

Isolation of *Actinomyces* Bacteriophage from Human Dental Plaque

CAROLYN A. TYLENDA,[1]* CHRISTI CALVERT,[1] PAUL E. KOLENBRANDER,[1] AND ANTHONY TYLENDA[2]

Microbiology Section, Laboratory of Microbiology and Immunology, National Institute of Dental Research, Bethesda, Maryland 20205,[1] and Department of Fixed Prosthodontics, School of Dentistry, Georgetown University, Washington, D.C. 20007[2]

Received 11 January 1985/Accepted 19 March 1985

Human dental plaque samples were screened for the presence of bacteriophage for *Actinomyces viscosus* and *Streptococcus sanguis*. None of the 336 samples yielded phage for *S. sanguis*, but 10 contained virulent actinomyces phage. A high host cell specificity was observed in that one phage isolate infected only *A. viscosus* T14V, eight phage isolates infected only *A. viscosus* MG-1, and one infected both strains. None was capable of productively infecting various other actinomyces strains that represented the six actinomyces coaggregation groups. Because phage-containing samples occurred randomly in this survey, no correlation between the individual collecting the samples, dental clinic, or type of patient and the presence of phage in the sample was noted. Examination of one of the samples that yielded phage for the presence of a natural host strain for that particular phage resulted in the isolation of two strains which were identified as *A. viscosus* serotype II and *Actinomyces naeslundii* serotype I. This is the first report of an *A. naeslundii* host strain and actinomyces bacteriophage of human dental plaque origin. The finding of both phage and host strains in the same dental plaque sample along with the observation of high host cell specificity by these phage provide indicators that support an active role for actinomyces bacteriophage in oral microbial ecology. The use of these freshly isolated phage as probes to study actinomyces coaggregation properties is discussed.

Bacteriophage for actinomyces were found previously only in sewage (5). Although many actinomyces have been tested as potential hosts, the four available phage productively infect a single strain, *Actinomyces viscosus* MG-1, a human oral isolate (19). In that same study, we found that these phage bind irreversibly to several other actinomyces. In fact, their ability to bind to certain reagent actinomyces strains was used to probe the surface for structures that mediate coaggregation. Bacteriophage-resistant mutants of *A. viscosus* MG-1 were isolated and shown to be altered in cell-to-cell recognition patterns with streptococcal coaggregation partners (19). The simultaneous loss of both the ability to bind phage and the ability to mediate certain coaggregations suggested that a common surface structure participated in both functions.

The potential utility of this approach prompted a search for new and different actinomyces phage. Human dental plaque was chosen as the source, and *A. viscosus* MG-1 was used as the indicator for the presence of phage. We report here the isolation of 10 phage and discuss their use in probing the actinomyces cell surface. Our studies with phage probes were conducted in an effort to uncover critical surface adhesins that are required for cell-cell or cell-solid surface interactions and that are involved integrally in the maturation of periodontal plaque.

MATERIALS AND METHODS

Bacterial strains and bacteriophage. *A. viscosus* strains MG-1 (5), T14V (4), PK455 (10), PK455-2 (obtained from John Cisar of the National Institute of Dental Research), PK1603, PK1643, PK1610, PK1623, PK1632, PK1648, PK1657, and PK1662 (19), *Actinomyces naeslundii* strains ATCC 12104, I, PK947, PK606, PK954, and PK990, *Actinomyces* sp. strain WVa-963 VPI D33C-25 (PK1259), and *Streptococcus sanguis* DL1 (Challis) (4, 13–15) were grown in TYNP medium as described earlier (19). Phage AV-1, AV-2, AV-3, and 1281 are of nonoral (sewage) origin and have been described previously (5, 19, 20). Bacteriophage AV-1 was propagated in *A. viscosus* MG-1. Phage titers were determined by the soft-agar overlay method (1).

Reconstruction experiments. To test the efficiency of our selection procedure for isolating new actinomyces phage, we examined a reconstruction of the experimental design by using phage AV-1. Of particular importance was stability of phage in transport medium (Trypticase soy broth [BBL Microbiology Systems, Cockeysville, Md.] containing 0.05% Tween-20 [Sigma Chemical Co., St. Louis, Mo.] and 0.2% gelatin [Nutritional Biochemicals Corp., Cleveland, Ohio]), efficiency of detection of phage in diluted samples of phage stocks in transport medium, effect of particulate and biological materials in human dental plaque on phage viability, and ability to detect phage after membrane filtration of phage-dental plaque suspensions. Phage stability was tested by incubating diluted (1 to 50 phage per 5 μl of transport medium) phage suspension at room temperature for 24 h and at 4°C for 7 days, conditions that approximated those used in the dental plaque collection regimen (see below). Phage viability was determined by spotting a portion of phage suspension onto a top-agar overlay plate containing *A. viscosus* MG-1, incubating it at 33°C for 24 h, and examining the spotted area for lysis of the indicator bacterium. Testing the effect of dental plaque material on phage viability was done by incubating pooled dental plaque from all surfaces of teeth in all quadrants of two individuals along with 200 AV-1 phage in 100 μl of transport medium. After 1 h at room temperature, the mixture was centrifuged at 6,500 × *g* for 10 min, and the phage titer in the supernatant was determined. The effect of membrane filtration was monitored with a 0.45-μm-pore-size Durapore filter (Millipore Corp., Bedford, Mass.).

Collection and processing of human dental plaque samples. A 250-μl volume of transport medium in 500-μl polypropylene microfuge tubes (Beckman Instruments, Inc., Palo Alto, Calif.) was sterilized by autoclaving. The tubes

* Corresponding author.

were given to dental hygienists at the National Institute of Dental Research dental clinic and the Commissioned Officers Dental Clinic, National Institutes of Health, and were dispensed to students at Georgetown University School of Dentistry. Tubes were kept at room temperature until dental plaque was added and then were stored at 4°C. Dental plaque that was removed from patients during routine scaling and root planing procedures was added to tubes containing sterile transport medium. The plaque was a mixture of supra- and subgingival plaque from all quadrants and surfaces. Tubes containing plaque were picked up once a week for processing. Each sample was vortexed vigorously for 60 s and centrifuged at 6,500 × *g* for 10 min. The supernatant fluid was passed through a 0.45-μm-pore-size Durapore filter, and 5 μl of each filtrate was spotted onto brain heart infusion (BHI) agar plates (Difco Laboratories, Detroit, Mich.; 1.5% agar) with top-agar overlays containing *A. viscosus* MG-1 or *S. sanguis* DL-1. In addition, 140 of the 336 samples were tested with *A. viscosus* T14V. Plates were incubated under anaerobic conditions at 33°C and examined after 24 h for lysis zones. When a lysis zone was detected, the top agar in that area was scraped from the plate, added to 500 μl of TYNP broth containing 0.05% Tween-20, and vortexed. The tubes were left for 1 h at room temperature to allow for elution of phage from the agar. After centrifugation, the supernatant fluid was filtered, diluted, and plated to obtain individual plaques. Phage was purified by three successive transfers and serial dilution of an eluate of an individual plaque.

The first five patients whose plaque was positive for actinomyces phage were recalled, and a second, and in one case a third, dental plaque sample was taken and examined for the presence of phage.

Field controls were also conducted. Phage AV-1 was added to some of the transport tubes (final concentration, 15 phage per 5 μl). Individuals collecting dental plaque were instructed to include one control tube for each week of the study (total length of study, 6 weeks). In addition to adding dental plaque from a patient to a tube containing transport medium, they put dental plaque from that patient into a control tube containing phage AV-1. The identity of the control tube was not made known to the investigator processing the sample until the results were tabulated.

Isolation of a human oral host strain. Dental plaque from one patient which was positive for the presence of actinomyces bacteriophage was serially diluted in TYNP broth and spread onto BHI agar plates. After 3 to 5 days of incubation at 33°C under anaerobic conditions, 100 colonies were selected, streaked onto BHI agar plates, and reincubated for 48 h. Each isolate was examined by two independent methods for its ability to serve as a host strain for phage isolated from that individual. With the spot-test lysis method, we tested each isolate as an indicator by adding cells to a top-agar overlay and spotting a 5-μl portion of phage lysate onto each plate. The second method involved spreading half of each BHI agar plate with a suspension of phage. Each isolate was streaked onto the plates in a direction such that half of the streak was on agar containing phage and half was on agar without phage. MG-1 was streaked down the center of each plate as a control, because the phage used in this experiment had been isolated on this strain and thus was virulent for it. Putative host strains were purified and retested by the spot-test lysis method.

Host range. Each phage isolate was examined by the spot-test lysis method for its ability to lyse several actinomyces strains. If the potential host strain tested positive by the spot-test lysis method, then serial dilutions of the phage were plated with this host as indicator. The strains tested included representative strains for each of the six coaggregation groups of actinomyces (4, 13, 14) and mutant classes I and II (spontaneously occurring bacteriophage-resistant mutants of *A. viscosus* MG-1 which exhibit unique sensitivity patterns to actinomyces bacteriophage AV-1, AV-2, AV-3, and 1281) (19, 20). *A. viscosus* PK455-2, a nonfimbriated mutant of strain T14V, was also tested.

Electron microscopy. Carbon-coated Formvar grids were floated for 1 min on a drop of phosphate-buffered physiological saline (20 mM phosphate, pH 7.2) placed on a lysis zone of phage CT2. Excess buffer was removed by capillary action. Phage were negatively stained with 2% unbuffered phosphotungstic acid. Preparations were examined with a JEOL 100-CX electron microscope at 80 kV.

RESULTS

Properties of phage selection procedure. To determine the appropriate conditions for isolating new actinomyces phage from human dental plaque, a series of experiments was performed with one of the known actinomyces phage, AV-1, and its indicator host strain, *A. viscosus* MG-1. These reconstruction experiments demonstrated that this phage could easily be detected in a fresh plaque sample. The level of sensitivity of the spot-test lysis method was one or two phage in a 5-μl volume of transport medium (Fig. 1). Removal of potentially contaminating bacteria was achieved by membrane filtration. One concern was that phage might not be detected because they would be bound to the membrane filter or to the dental plaque particulate material, but filtration of dilute suspensions of AV-1 (20 phage per 10 μl) with or without addition of dental plaque showed no evidence of loss of phage (Fig. 2). Finally, to test phage stability

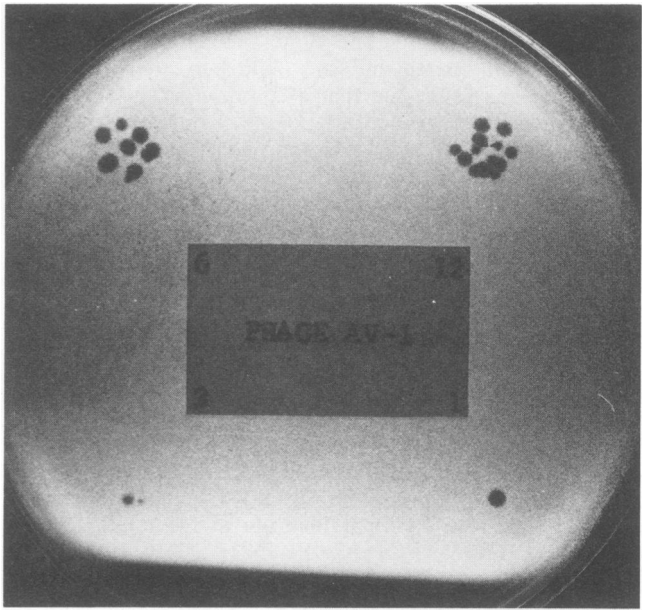

FIG. 1. Sensitivity of the spot-test lysis assay for detecting phage. A suspension of phage AV-1 was spotted onto a top-agar overlay containing *A. viscosus* MG-1 and incubated for 24 h at 33°C. The number of expected phage in the 5-μl volume of the spotted dilution sample is shown in the corners of the rectangle; it was calculated from the titer of phage in the undiluted stock suspension.

in the transport medium, we stored control tubes containing a low titer of phage AV-1 (10 to 20 phage per 5 μl) at 4°C for up to 6 weeks. Each of the 15 control tubes used throughout the 6-week duration of this study tested positive for phage (data not shown). Thus, the indicator host for actinomyces phage of sewage origin successfully detected very low numbers of AV-1 under the conditions chosen to select for human oral actinomyces bacteriophage.

Isolation of bacteriophage from human dental plaque. Ten of 336 dental plaque samples tested yielded virulent phage for actinomyces. None of the 336 sample filtrates produced visible lysis zones on *S. sanguis* DL-1. Of the 10 phage-positive patients, 5 were seen at the National Institute of Dental Research dental clinic, which contributed 111 plaque samples, and 5 were seen at Georgetown University School of Dentistry, which contributed 209 samples. The remaining 16 samples were obtained from the Commissioned Officers Dental Clinic, National Institutes of Health.

Nine of the 10 phage were isolated with *A. viscosus* strain MG-1 as the indicator microorganism. The initial spotted area ranged in appearance from approximately 100 individual poorly defined plaques (Fig. 3A) to a fully clear lysis zone equal in area to that of the original 5-μl spot (Fig. 3C). In each case, an eluate of the lysis area produced a clear lysis zone on a top agar overlay of strain MG-1 (data not shown), and, in addition, was shown when serially diluted to be capable of producing individual plaques in an MG-1 lawn (Fig. 3B).

Five phage-positive patients were resampled. The presence of virulent phage for strain MG-1 was found in four of the five cases. An example is shown in Fig. 3C. The four phage-containing plaque samples were taken from 9 to 19 days after the initial sampling. Dental plaque from one of the four patients was sampled a third time, 14 days after the second sampling, and it again tested positive. A second plaque sample from the fifth individual who tested positive for phage was taken 34 days after the initial sampling, and it tested negative on MG-1.

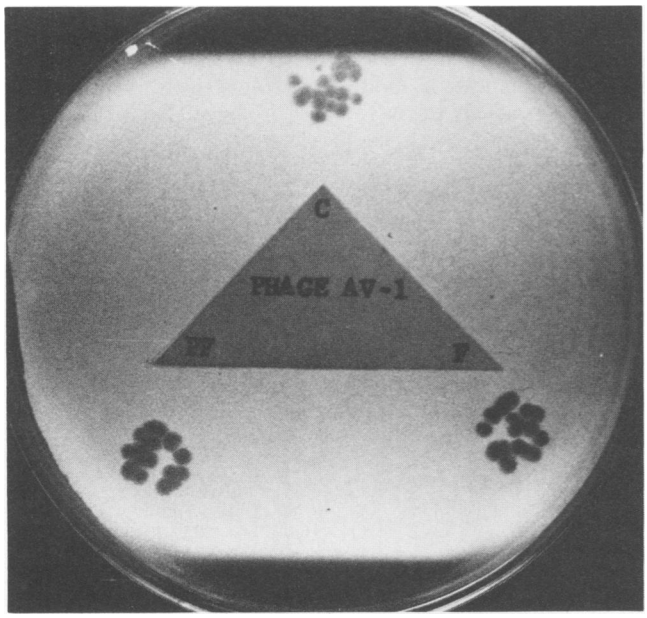

FIG. 2. Effect of filtration and the addition of dental plaque on ability to detect phage. An unfiltered suspension of phage AV-1 was spotted onto an overlay plate containing *A. viscosus* MG-1 (C). A portion of phage suspension was passed through a 0.45-μm-pore-size Durapore filter before spotting (F). To another portion, dental plaque from two individuals was added, incubated, centrifuged, and filtered before spotting (PF). A 5-μl sample was spotted from each suspension.

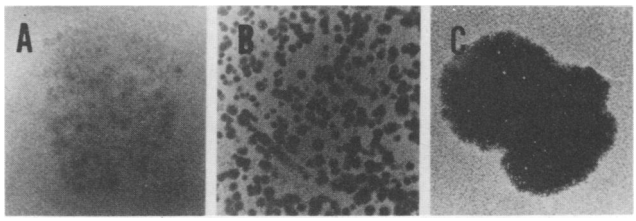

FIG. 3. Appearance of lysis zones after plating dental plaque samples on *A. viscosus* MG-1. (A) Lysis zone produced by the initial filtrate of dental plaque from one patient. (B) Individual plaques produced when the serially diluted eluate of the initial lysis zone was plated. (C) Lysis zone produced by the filtrate of the second dental plaque sample (taken 19 days after the initial sampling) from the same patient. The phage purified from this patient was later designated phage CT1.

One phage (CT8) was isolated from a plate containing *A. viscosus* T14V as indicator. The original lysis zone consisted of about 40 small, indistinct plaques.

Host range. Of the nine phage isolated on MG-1, only phage CT7 was also virulent for T14V (Table 1). Phage CT7 showed no greater plating efficiency for MG-1 when compared with T14V. However, the plaques produced with T14V as host were smaller and less well defined. Phage CT8, which was selected with *A. viscosus* T14V, was not virulent for MG-1. Both phage CT7 and CT8 were also virulent for *A. viscosus* PK455-2, a nonfimbriated mutant derived from T14V. None of the oral actinomyces phage lysed class I or class II mutants (data not shown), which are derivatives of MG-1 and resistant to all four (class I) or just AV-3 (class II) actinomyces bacteriophage of nonoral origin (20).

A. viscosus and *A. naeslundii* reagent strains that are

TABLE 1. Host range of actinomyces bacteriophage of oral and nonoral origin with *A. viscosus* MG-1, T14V, and CT1002 and *A. naeslundii* CT1001 isolated from human dental plaque[a]

Phage origin	Lysis of strain			
	MG-1	T14V	CT1001	CT1002
Human dental plaque				
CT1	+	−	−	+
CT2	+	−	−	−
CT3	+	−	−	−
CT4	+	−	+	−
CT5	+	−	+	−
CT6	+	−	−	−
CT7	+	+	−	−
CT8	−[b]	+	−[b]	−[b]
CT9	+	−	−	−
CT10	+	−	−	−
Sewage				
AV-1	+	−	−	−[b]
AV-2	+	−	−	−[b]
AV-3	+	−	−	−
1281	+	−	−	−[b]

[a] The spot-test lysis method outlined in the text was used.

[b] A lysis zone was seen by using the spot-test lysis method. However, plating of serial dilutions of phage on the host strain did not yield phage plaques.

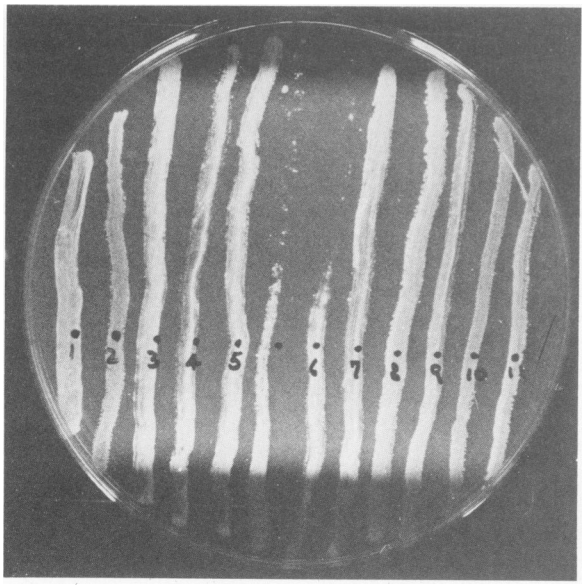

FIG. 4. Isolation of a host strain for phage CT1. Isolated colonies from spread plates of serially diluted dental plaque from the patient from whom phage CT1 was isolated were streaked onto BHI plates. Before streaking was done, the upper half of the plates were spread with a suspension of phage CT1. The center streak (unnumbered) is *A. viscosus* MG-1, the indicator strain used to isolate phage CT1. Lysis in the upper half of the plate indicates sensitivity to the phage.

classified into six coaggregation groups on the basis of their ability to coaggregate with oral streptococci were also tested as potential host strains. Actinomyces coaggregation group A, represented by *A. viscosus* strains MG-1 and T14V, was discussed above. None of the phage was virulent for coaggregation group B actinomyces, represented by *A. naeslundii* strains I and ATCC 12104; group C, represented by *A. naeslundii* PK947; group D, represented by *A. naeslundii* PK606; group E, represented by *A. naeslundii* strains PK954 and PK990; or group F, represented by *A. naeslundii* PK1259 (data not shown). In several of the above cases, lysis zones were seen by using the spot-test lysis method. However, plating serial dilutions of the phage did not produce plaques. The cause of the lysis zones was not investigated further.

Identification and properties of two new host strains for oral actinomyces phage. A strain (CT1002) which will support the productive infection of bacteriophage CT1 was isolated from dental plaque of the same patient from whom the phage was isolated (strain 6, Fig. 4). Based on the tests and the identification key outlined in the *Anaerobe Laboratory Manual* (8) and on fluorescent antibody reactions (kindly done by the staff at the Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg), this strain was identified as *A. viscosus* serovar II. It was not sensitive to infection by the other phage of dental plaque origin or by the phage of nonoral origin (Table 1).

Another bacterial strain, CT1001, identified as *A. naeslundii* serovar I, was isolated from the same dental plaque sample as strain CT1002. *A. naeslundii* CT1001 did not support a productive infection by phage CT1 but was sensitive to infection by phage CT4 and CT5 (Table 1). None of the other oral or nonoral phage infected CT1001.

Electron microscopy. An electron micrograph of phage CT2 (Fig. 5) revealed a structure similar in size and morphology to nonoral phage AV-1 (5). The phage appeared to have a polyhedral head and a short tail.

DISCUSSION

Reports of bacteriophage which can infect oral bacteria are rare. *Actinobacillus actinomycetemcomitans* 651, a strain isolated from a juvenile periodontitis patient, contains a prophage (17). Similarly, a prophage was found in *Streptococcus mutans* PK1 (7). In both of the above cases, mitomycin C was used successfully to induce phage production. Virulent phage for *Veillonella* strains have been found in the oral cavity (16, 18). In one study, examination of oral washings from 200 patients yielded 25 virulent phage for *Veillonella* strains. The phage were classified into two groups based on plaque morphology and serology. Observations under an electron microscope showed major differences in morphology between the two groups (18).

Perhaps the primary significance of this study is that actinomyces phage appear to be resident constituents of the oral cavity. Phage could be repeatedly detected in the dental plaque of individuals shown on the initial sampling to harbor phage. Besides phage, host strains for these phage were found. Because this was not an exhaustive study to detect either host strains or phage, it is probable that a more intensive investigation will reveal a larger number of both. Indeed, several investigators have observed phagelike particles in electron micrographs of dental plaque (2, 6, 9). The phage that were isolated appeared randomly among the dental plaque samples. No correlation with any of the variables in this survey was noted. These variables included the person taking the plaque sample, the type of dental clinic, and age, sex, or state of health of the patient. Thus, actinomyces phage may be a common occurrence in the human oral cavity.

Only three laboratory strains were used in the screening for phage in dental plaque samples, and phage were found for two of the strains. The phage showed a narrow host specificity, which suggests that phage would be detected at a higher frequency if additional strains were included as potential hosts in the screening protocol. An example of

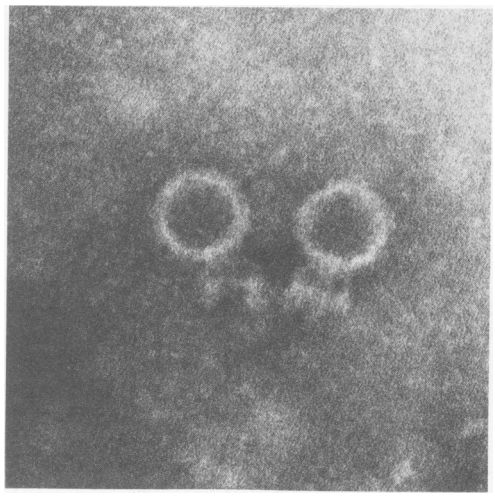

FIG. 5. Electron micrograph of bacteriophage CT2 (×256,000). The phage were eluted from a spot lysis zone directly onto a carbon-coated copper grid and stained with 2% phosphotungstic acid.

phage specificity was found in the current study when we isolated an *A. naeslundii* host, strain CT1001 (Table 1). This is the first *A. naeslundii* strain known to be a host for actinomyces phage. It was surprising that it was not infected by phage CT1, because both were isolated from the same dental plaque sample. However, this strain was a host for phage CT4 and CT5, which were obtained from two other samples. These observations suggest that if strain CT1001 was used in a similar screening for new phage in dental plaque, some of the samples would yield phage for CT1001.

Our observations indicate that specificity is clearly evident in host strains as well as in phage. Both of the new hosts and strain T14V supported productive infections by only one or at most two phage. The fourth host, MG-1, served as the indicator organism for the screening procedure and was infected by all but phage CT8, which was selected with T14V (Table 1). The causes of the permissive or restrictive phenotypes of these actinomyces are unknown. One possibility is that MG-1 possesses a more permissive restriction-modification system for foreign DNA than does T14V, CT1001, or CT1002. Alternatively, MG-1 may express certain surface receptors for phage that are not synthesized by other actinomyces. Both possibilities are of great interest and are currently under investigation.

Both new host strains exhibit the actinomyces coaggregation group A pattern, as do *A. viscosus* MG-1 and T14V, but there does not appear to be any correlation between being a phage host and exhibiting properties of coaggregation group A. It is important to consider, however, that all members of coaggregation group A are capable of lactose-reversible coaggregation with certain streptococci, and the surface structure that mediates this kind of coaggregation is the type 2 fimbriae on actinomyces (3). Coaggregation defective mutants of *A. viscosus* T14V, PK455 (lacks type 2 fimbriae) and PK455-2 (lacks both type 1 and type 2 fimbriae), remain sensitive to the same phage (CT7 and CT8) that infect T14V (data not shown). Thus, the phage receptors on the actinomyces surface do not appear to be associated with either fimbriae. Moreover, phage bind equally well to heated and unheated cells, which indicates that the phage receptor is heat stable and which supports the observed indifference of the phage to heat-labile fimbriae.

One of the goals of this study was to increase the number of actinomyces phage available to use as probes for studies of cell surface coaggregation receptors. The variation in host range with respect to strains MG-1, T14V, CT1001, and CT1002 indicates that at least 5 of the 10 new phage isolates are not identical. None of the oral phage infected class II mutants of MG-1 (data not shown), which distinguishes them from the nonoral AV-1, AV-2, and 1281 (19). Only AV-3 could be identical to the oral phage (CT2, CT3, CT6, CT9, or CT10), but this also is unlikely because there are subtle differences in plaque appearance and plaque development in the soft-agar overlay (unpublished observations).

Further studies with these oral and nonoral phage are directed at the nature of cell surface receptors on actinomyces. Of special interest is the relationship of cell surface structures to cell adhesion properties. For example, all four hosts are members of coaggregation group A, yet they were quite different in their phage sensitivity patterns (Table 1), which in turn reflect variations in cell surface phage receptors. Phage can be used as probes to define these differences in the cell surfaces of each strain, and phage-resistant mutants may be altered in the coaggregation pattern characteristic of coaggregation group A. In fact, phage-resistant mutants of MG-1 were used to demonstrate differences in the cell surface of MG-1 and T14V (11). It should be noted that actinomyces coaggregate with many other kinds of oral bacteria besides streptococci, including *Bacteroides* spp. (12), *Capnocytophaga* spp. (10a), and some fusobacteria, veillonella, and selenomonads (P. E. Kolenbrander, R. N. Andersen, and L. V. Holdeman, unpublished data). Thus, bacteriophage-resistant mutants of any of these four actinomyces would be useful in the identification of these heretofore undetectable surface components that may have the dual function of phage receptor and mediator of coaggregation with one or more of the parental partners.

ACKNOWLEDGMENTS

We thank Julie Haller and Shelley Harlow of the National Institute of Dental Research dental clinic and Wanda Dixon of the Commissioned Officers Dental Clinic, NIH, for their help in conducting this study. We thank Arthur Hand, Chief, Laboratory of Oral Biology and Physiology, National Institute of Dental Research, for the electron micrograph.

LITERATURE CITED

1. **Adams, M. H.** 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. **Brady, J. M., W. A. Gray, and M. A. Caldwell.** 1977. The electron microscopy of bacteriophage-like particles in dental plaque. J. Dent. Res. **56**:991–993.
3. **Cisar, J. O., E. L. Barsumian, S. H. Curl, A. E. Vatter, A. L. Sandberg, and R. P. Siraganian.** 1981. Detection and localization of a lectin on *Actinomyces viscosus* T14V by monoclonal antibodies. J. Immunol. **127**:1318–1322.
4. **Cisar, J. O., P. E. Kolenbrander, and F. C. McIntire.** 1979. Specificity of coaggregation reactions between human oral streptococci and strains of *Actinomyces viscosus* or *Actinomyces naeslundii*. Infect. Immun. **24**:742–752.
5. **Delisle, A. L., R. K. Nauman, and G. E. Minah.** 1978. Isolation of a bacteriophage for *Actinomyces viscosus*. Infect. Immun. **20**:303–306.
6. **Halhoul, N., and J. R. Colvin.** 1975. Virus-like particles in association with a microorganism from human gingival plaque. Arch. Oral Biol. **20**:833–836.
7. **Higuchi, M., G. H. Rhee, S. Araya, and M. Higuchi.** 1977. Bacteriophage deoxyribonucleic acid-induced mutation of *Streptococcus mutans*. Infect. Immun. **15**:938–944.
8. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
9. **Kerebel, B., S. Clergeau-Guerithault, and P. Forlot.** 1975. Etude ultrastructurale de plaques microbiennes dentaires chez des sujets idemnes de carie. Ann. Microbiol. (Paris) **126A**:203–229.
10. **Kolenbrander, P. E.** 1982. Isolation and characterization of coaggregation-defective mutants of *Actinomyces viscosus*, *Actinomyces naeslundii*, and *Streptococcus sanguis*. Infect. Immun. **37**:1200–1208.

10a. **Kolenbrander, P. E., and R. N. Andersen.** 1984. Cell to cell interactions of *Capnocytophaga* and *Bacteroides* species with other oral bacteria and their potential role in development of plaque. J. Periodontal Res. **19**:564–569.

11. **Kolenbrander, P. E., and R. N. Andersen.** 1985. Use of coaggregation-defective mutants to study the relationship of cell-to-cell interactions and oral microbial ecology, p. 164–171. *In* S. E. Mergenhagen and B. Rosan (ed.), Molecular basis of oral microbial adhesion. American Society for Microbiology, Washington, D.C.
12. **Kolenbrander, P. E., R. N. Andersen, and L. V. Holdeman.** 1985. Coaggregation of oral *Bacteroides* with other bacteria: central role in coaggregation bridges and competitions. Infect. Immun. **48**:741–746.
13. **Kolenbrander, P. E., Y. Inouye, and L. V. Holdeman.** 1983. New

Actinomyces and *Streptococcus* coaggregation groups among human oral isolates from the same site. Infect. Immun. **41**:501–506.

14. **Kolenbrander, P. E., and B. L. Williams.** 1981. Lactose-reversible coaggregation between oral actinomycetes and *Streptococcus sanguis*. Infect. Immun. **33**:95–102.
15. **Kolenbrander, P. E., and B. L. Williams.** 1983. Prevalence of viridans streptococci exhibiting lactose-inhibitable coaggregation with oral actinomycetes. Infect. Immun. **41**:449–452.
16. **Shimizu, Y.** 1968. Experimental studies on the bacteriophages of the *Veillonella* strains isolated from the oral cavity. Odontology (Tokyo) **55**:533–542.
17. **Stevens, R. H., B. F. Hammond, and C. H. Lai.** 1982. Characterization of an inducible bacteriophage from a leukotoxic strain of *Actinobacillus actinomycetemcomitans*. Infect. Immun. **35**:343–349.
18. **Totsuka, M.** 1976. Studies on veillonella phages isolated from washings of human oral cavity. Bull. Tokyo Med. Dent. Univ. **23**:261–273.
19. **Tylenda, C. A., E. Enriquez, P. E. Kolenbrander, and A. L. Delisle.** 1985. Simultaneous loss of bacteriophage receptor and coaggregation mediator activities in *Actinomyces viscosus* MG-1. Infect. Immun. **48**:228–233.
20. **Tylenda, C. A., P. E. Kolenbrander, and A. L. Delisle.** 1983. Use of bacteriophage-resistant mutants to study *Actinomyces viscosus* cell surface receptors. J. Dent. Res. **62**:1179–1181.