each core and is 0.10 per mil for $\delta^{18}O$ and 0.10 per mil for $\delta^{13}C$.

15. *Globorotalia tumida* was analyzed for $\delta^{18}O$ in samples V28-179 and DSDP 573 because carbonate dissolution has eliminated shallow-dwelling species such as *G. sacculifer*. C. G. Adelseck and T. F. Anderson [*Geology* **6**, 388 (1978)] showed that $\delta^{18}O$ stratigraphy based on *G. tumida* correlates well with other late Pleistocene isotope stratigraphy.
16. Magnetostratigraphy and biostratigraphy are given in the following publications: for V28-179, see (*6*); J. Backman and N. J. Shackleton, *Mar. Micropaleontol.* **8**, 141 (1983); for DSDP 502, D. V. Kent and D. Spariosu, *Init. Rep. Deep Sea Drill. Proj.* **68**, 419 (1982); L. D. Keigwin, Jr., *ibid.*, p. 269; for DSDP 572 and 573, W. L. Prell, *Init. Rep. Deep Sea Drill. Proj.*, in press (DSDP 572 was correlated to DSDP 573, which has the magnetostratigraphic record, by detailed carbonate stratigraphy); for V20-163, J. D. Hays *et al.*, *Geol. Soc. Am. Bull.* **80**, 1481 (1969); J. Backman and N. J. Shackleton, *Mar. Micropaleontol.* **8**, 141 (1983).
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Shiga-Like Toxin–Converting Phages from *Escherichia coli* Strains That Cause Hemorrhagic Colitis or Infantile Diarrhea

Abstract. Escherichia coli *K-12 acquired the ability to produce a high titer of Shiga-like toxin after lysogenization by either of two different bacteriophages isolated from a highly toxinogenic* Escherichia coli *0157:H7 strain that causes hemorrhagic colitis. One of these phages and another Shiga-like toxin–converting phage from an* Escherichia coli *026 isolate associated with infantile diarrhea were closely related in terms of morphology, virion polypeptides, DNA restriction fragments, lysogenic immunity, and heat stability, although a difference in host range was noted. These phages are currently the best-characterized representatives from a broader family of Shiga-like toxin–converting phages.*

Scotland *et al.* (*1*) described a toxin-converting bacteriophage released spontaneously from a plasmid-negative *Escherichia coli* K-12 strain that had become toxinogenic after cocultivation with *E. coli* 026 strain H-19. Smith *et al.* (*2*) isolated two different converting phages, H-19A and H-19B, that were released spontaneously from *E. coli* H-19. They also isolated converting phages from several other *E. coli* isolates. *Escherichia coli* H-19, which caused an outbreak of infantile diarrhea in Great Britain more than 15 years ago, is noninvasive and does not produce heat-labile or heat-stable enterotoxin (*3*). It makes large amounts of a cytotoxin that appears to be the same as the *Shigella dysenteriae* 1–like (Shiga-like) toxin (*4*, *5*). Shiga-like toxin is produced in various amounts by some strains of *E. coli* and is defined by its ability to be neutralized by antibodies against purified Shiga toxin (*6*). Shiga-like toxin is cytotoxic for Vero cells and was originally called Vero cell cytotoxin (*1*, *2*, *4*, *5*).

We report that *E. coli* 0157:H7 strain 933, which causes hemorrhagic diarrhea and produces large amounts of Shiga-like toxin, also harbors two different toxin-converting phages designated 933J and 933W. We present morphological, biochemical, and genetic evidence that converting phage 933J from strain 933 is closely related to phage H-19A and provide data indicating that a family of Shiga-like toxin–converting phages exists in *E. coli* strains in nature.

Excherichia coli of the serotype 0157:H7 was recently reported as being a causative agent of hemorrhagic colitis in the United States (*7*) and Canada (*8*) and has been isolated from feces of patients with the hemolytic-uremic syndrome (*9*). It has been suggested that these geographically diverse isolates represent a single, widely dispersed clone of virulent *E. coli* (*10*). Riley *et al.* (*11*) characterized several *E. coli* 0157:H7 strains that were obtained from hamburger and from diarrheal stools of patients during investigation of two food-borne outbreaks of hemorrhagic colitis in the United States. They found that none of the *E. coli* 0157:H7 isolates were enteroinvasive and that none elaborated *E. coli* heat-labile toxin (LT) or *E. coli* heat-stable toxin (ST). The virulence of *E. coli* 0157:H7 for animals was demonstrated by Farmer *et al.* (*12*), who showed that infant rabbits developed diarrhea when fed *E. coli* 0157:H7. The possibility that a toxin other than LT or ST might be involved in the pathogenesis of *E. coli* 0157:H7 diarrheal disease was suggested by Johnson *et al.* (*13*). They reported that Canadian isolates of *E. coli* 0157:H7 made a cytotoxin, which O'Brien *et al.* (*5*) subsequently found could be completely neutralized by antitoxin to purified Shiga toxin. The level of Shiga-like toxin made by three 0157:H7 isolates examined by O'Brien *et al.* was equivalent to that of *Shigella dysenteriae* 1, or 10^5 to 10^6 50 percent cytotoxic doses (CD_{50}) per milliliter of culture supernatant and $\geq 10^6$ CD_{50} per milligram of protein in lysates of bacteria. In a separate study, O'Brien *et al.* (*14*) purified Shiga-like toxin from one of the *E. coli* 0157:H7 strains (designated 933) to homogeneity by the same procedure previously used to purify toxin from *E. coli* 026 strain H30 (*15*). The pure toxins from *E. coli* 0157:H7 strain 933, *E. coli* 026 strain H30, and *Shigella dysenteriae* 1 strain 60R had the same subunit structure and the same biological activities (*14*).

We developed methods to induce phages from *E. coli* strains H-19 and 933 and to optimize conditions for performing plaque assays for the phages. Bacterial cultures (5 ml) were grown to an optical density of 0.5 (wavelength, 600 nm) in LB broth (*16*) prepared with half the usual amount of NaCl and supplemented with 10 m*M* $CaCl_2$ and 0.001 percent thiamine. Cells were then harvested by centrifugation, resuspended in 5-ml samples of 10 m*M* $CaCl_2$, and irradiated (40 J/m^2) in 100-mm glass petri dishes. The irradiated bacteria were diluted tenfold in modified LB broth and then incubated for 5 hours at 37°C in foil-

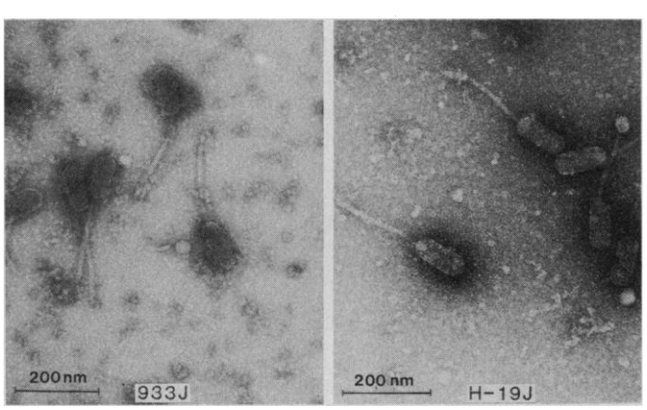

Fig. 1. Electron micrographs of plaque-purified H-19J and 933J phages negatively stained with uranyl acetate (*p*H 4.5).

covered tubes. After adding two drops of chloroform to each tube and removing debris by low-speed centrifugation, we sterilized the lysates by membrane filtration and stored them at 4°C. *Escherichia coli* 395-1 (*17*), a substrain of *E. coli* K-12, was used as the indicator strain for plaque assays and as the host strain in phage conversion experiments. Samples containing tenfold serial dilutions of phage in modified LB broth were adsorbed onto indicator cells (optical density, 0.5 at 600 nm) in modified LB broth for 20 minutes at 37°C. Portions (100 μl) from the absorption mixtures were plated in 3 ml of modified LB soft agar (0.7 percent) and poured into petri dishes containing modified LB hard agar (1.5 percent). Plaques were counted after the plates were incubated overnight at 37°C. Lysates of strain H-19 typically contained more infectious phage [10^9 plaque-forming units (PFU) per milliliter] than lysates of strain 933 (10^5 PFU/ml).

Next we assessed whether the phages released from *E. coli* strains H-19 and 933 after induction with ultraviolet light were also toxin-converting. For this purpose, 50 large plaques from lysates of each strain were excised from the assay plates with Pasteur pipettes, inoculated into 1-ml samples of Penassay broth (Difco), and incubated overnight at 37°C. Each culture supernatant was diluted 1:250 and 100-μl samples were tested on HeLa cells for cytotoxicity (*18*). Each of the 50 cultures inoculated with material from single plaques of H-19 or 933 phage on lawns of *E. coli* K-12 substrain 395-1 contained detectable toxin. HeLa cell assay controls included undiluted supernatants from the nonlysogenized host strain *E. coli* 395-1 (not cytotoxic) and 1:250 dilutions of supernatants from *E. coli* H-19 or *E. coli* 933 (cytotoxic). One phage induced from *E. coli* H-19, designated phage H-19J, and one phage induced from *E. coli* 933, designated phage 933J, were then subjected to repeated single-plaque purifications, and stable derivatives of *E. coli* 395-1 and *E. coli* C600 lysogenized with each of the plaque-purified phages were prepared. The phage titers after ultraviolet induction of the two *E. coli*: 395-1 lysogens were the same (10^7 PFU/ml). Moreover, each of the *E. coli* 395-1 lysogens produced titers of Shiga-like toxin equivalent to the original *E. coli* H-19 and 933 strains (10^6 CD_{50} per milligram of protein in cell lysates and 10^5 CD_{50} per milliliter in supernatants when grown in deferrated glucose-syncase medium). In contrast, control cultures of *E. coli* K-12 substrain 395-1 or C600 made only trace levels of Shiga-like toxin under similar conditions [$\leq 10^2$ CD_{50} per milligram of protein in cell lysates and no detectable toxin in culture filtrates (*6, 19*)]. Hence the phage isolates 933J and H-19J both converted *E. coli* K-12 to produce high titers of Shiga-like toxin.

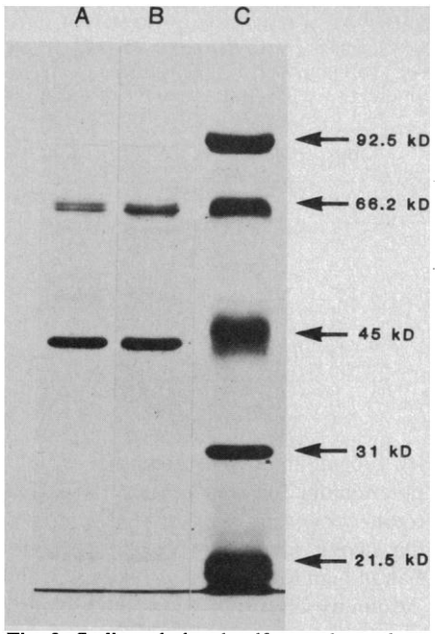

Fig. 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of polypeptides from plaque-purified, CsCl gradient–isolated converting phages H-19J (lane A) and 933J (lane B). Lane C contains a mixture of molecular weight standards (phosphorylase B, 92.5 kD; bovine serum albumin, 66.2 kD; ovalbumin, 45 kD; carbonic anhydrase, 31 kD; soybean trypsin inhibitor, 21.5 kD). Samples of phage were treated with SDS-containing buffer, subjected to electrophoresis in a 12 percent gel, and stained with Coomassie blue (*15*).

We then compared the morphology, polypeptides, and DNA restriction fragments of these two toxin-converting phages. High-titered phage stocks (10^{10} per milliliter) were prepared from pages harvested from soft agar overlays. Next the phages were purified by centrifugation through CsCl step gradients (*20*). The morphologies of the uranyl acetate–stained H-19J and 933J phages were indistinguishable by electron microscopy (Fig. 1). When the polypeptides of the two phages were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), the mobilities of their structural polypeptides were also the same (Fig. 2). Restriction endonuclease (EcoRI, Hind III, or Bam HI) digests of DNA extracted from the two phages were analyzed by agarose gel electrophoresis, and no differences were found (Fig. 3). Furthermore, ^{32}P-labeled H-19J phage DNA was hybridized in Southern blots with all restriction fragments of phage 933J DNA produced by digestion with EcoRI. These comparative studies of morphology, structural polypeptides, and DNA demonstrate that the Shiga-like toxin–converting phages 933J and H-19J are the same or are very closely related.

Additional studies were performed to determine the relation of phages 933J and H-19J to phages H-19A and H-19B (*2*) and to phage 933W, a toxin-converting phage released spontaneously from *E. coli* 933 and isolated by one of us (H.W.S.). These phages are representative of isolates collected on several different occasions from *E. coli* strains H-19 and 933 by the methods described above. Tests were performed to determine the specificity of lysogenic immunity conferred by these phages (Table 1). All the phages except 933W were stable to heating at 58°C for 30 minutes; the titer of 933W was decreased about 1000 times under these conditions. We drew the following conclusions:

1) H-19A and H-19J are independent isolates of the same phage; H-19B is a different phage. Both H-19A/J and H-

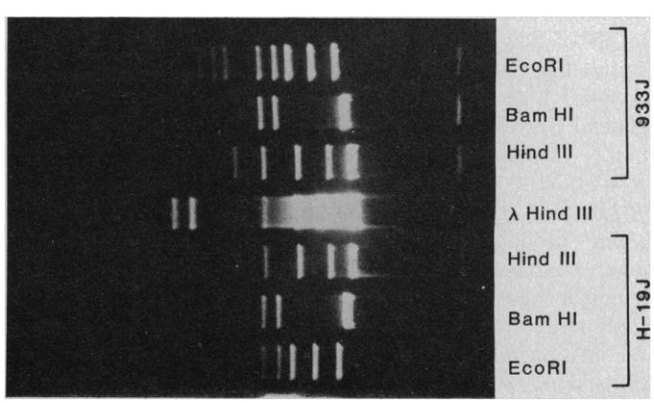

Fig. 3. Agarose gel electrophoresis and ethidium bromide staining of restriction endonuclease – digested DNA (*20*) from plaque-purified, CsCl gradient–isolated phages H-19J and 933J. The size of the intact DNA from each of the bacteriophages was estimated as 47 kilobase pairs on the basis of the sum of the sizes of the individual restriction fragments. The sizes of the fragments were determined by their relative electrophoretic mobilities when compared to Hind III reference fragments of DNA from coliphage .

Table 1. Capacity of different plaque-purified Shiga-like toxin–converting phages to form plaques on various *E. coli* hosts. Phage stocks were diluted 10^{-2} and plated in a soft agar overlay. The designations C600(H-19J), C600(H-19A), C600(933J), C600(H-19B), and C600(933W) indicate *E. coli* K-12 substrain C600 lysogenized with the phage H-19J, H-19A, 933J, H-19B, or 933W, respectively. Symbols: (+) formation of a large number ($>10^4$) of plaques on the host lawn after overnight incubation, (−) no plaques, and (*) less than 50 plaques.

Host strain of *E. coli*	Phage				
	H-19J	H-19A	933J	H-19B	933W
C600	+	+	+	+	+
C600(H-19J)	−	−	−	−	+
C600(H-19A)	−	−	−	−	+
C600(933J)	−	−	−	−	+
C600(H-19B)	+	+	+	−	+
C600(933W)	+	+	*	+	−

19B are converting phages that determine production of high titers of Shiga-like toxin (*2*).

2) *Escherichia coli* 933 also contains two different Shiga-like toxin–converting phages. Phage 933J is closely related to phage H-19A/J, as judged by similarities in morphology, virion proteins, restriction endonuclease fragments of genomic DNA, DNA-DNA hybridization in Southern blots, immunity specificity, and heat stability. Nevertheless, the restriction of plaquing of phage 933J but not H-19A/J on lawns of C600(933W) demonstrates that they are not identical.

3) A family of Shiga-like toxin–converting phages exists in nature. The distribution of members of this family and the extent of relatedness among them is not fully established and requires further study. We recently observed that ^{32}P-labeled DNA from phage H-19J hybridizes in Southern blots with specific restriction fragments of genomic DNA from selected strains of *E. coli* 0145, 0111, and 026 associated with diarrheal disease in humans. This finding suggests that phages belonging to the family of Shiga-like toxin–converting phages are also present in these strains of *E. coli*.

How the converting phages from *E. coli* 933 and H-19 control production of Shiga-like toxin has not yet been established. Because *E. coli* K-12 makes low levels of Shiga-like toxin (*6, 19*), it appears that the genome of *E. coli* K-12 contains the toxin structural gene or genes. However, lysogenization of *E. coli* K-12 with a toxin-converting phage results in a dramatic increase (up to 10,000-fold) in the amount of Shiga-like toxin produced. Converting phages could contain either the toxin structural genes or regulatory elements that act on toxin structural genes already present in the host bacterium. To determine which of these possibilities is correct, it will be necessary to isolate and characterize the toxin-converting genes from phage DNA by recombinant DNA techniques. The potential medical relevance of Shiga-like toxin–converting phages is suggested by the strong correlation between production of high levels of Shiga-like toxin and the ability of *E. coli* to produce bloody diarrhea or hemorrhagic colitis in humans (*19*), the presence of converting phages in such clinical isolates of *E. coli*, and the ability of the converting phages to determine high levels of toxin production in *E. coli* K-12 (*21*).

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21. After our manuscript was submitted for publication, H. R. Smith, N. P. Day, S. M. Scotland, J. R. Gross, and B. Rowe [*Lancet* **1984-II**, 242 (1984)] also reported the existence of Shiga-like toxin–converting phages in *E. coli* strains of serogroup 0157.
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Molecular Weight Determinations of Proteins by Californium Plasma Desorption Mass Spectrometry

Abstract. *The plasma desorption mass spectrometry method is used to determine the molecular weights of larger molecules than before, to determine the molecular weights of proteins and peptides in mixtures, and to monitor protein modification reactions. Proteins up to molecular weight 25,000 can now be studied with a mass spectrometric technique. Protein-peptide mixtures that could not be resolved with conventional techniques were successfully analyzed by this technique. The precision of the method is good enough to permit one to follow the different steps in the conversion of porcine insulin to human insulin.*

Molecular weight (MW) determination is normally one of the first steps in characterizing unknown protein molecules. The present commonly used techniques, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and gel chromatography, allow a precision of 10 to 20 percent, and the MW determination may be influenced by other properties of the molecule such as conformational state and hydrophobicity. For smaller molecules mass spectrometry (MS) is the method of choice. In the last few years methods based on