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Isolation of Bacteriophages Specific for the K1 Polysaccharide Antigen of *Escherichia coli*

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Five bacteriophage stocks were prepared after enrichment of a sewage sample using *Escherichia coli* O2:K1:H4 (strain U9/41). The bacteriophages were tested for their ability to lyse 224 strains of *E. coli* that had been tested for the presence of the K1 antigen by means of an antiserum-agar diffusion technique, using a meningococcus group B antiserum known to detect the *E. coli* K1 antigen. The standard test strains for *E. coli* K antigens 2 to 99 were used as control strains. Of the 101 strains found to possess the K1 antigen using the antiserum-agar technique, 93 were lysed by at least one of the bacteriophages, whereas 8 of the 123 strains apparently lacking K1 were lysed by one or more of the bacteriophages. None of the standard test strains for K antigens 2 to 99 was lysed by any of the bacteriophages. The eight strains thought to lack K1 but that were lysed by bacteriophage were re-examined by immunoelectrophoresis, using meningococcus group B antiserum; five of the eight strains gave a precipitin line corresponding to K1. The use of K1-specific bacteriophages offers an inexpensive and easy method for the identification of the K1 antigen.

Studies of neonatal meningitis have shown that when the etiological agent is *Escherichia coli* the strains frequently possess the K1 capsular polysaccharide antigen (1, 5, 6). The ability to identify this antigen is of importance in the study of the epidemiology and pathogenesis of the disease and may be useful in the clinical laboratory.

There is close antigenic similarity between the *E. coli* K1 antigen and the polysaccharide antigen of *Neisseria meningitidis* group B (2). In most studies so far the K1 antigen has been identified by means of an antiserum-agar technique, using a meningococcus group B antiserum (6). Although this method is convenient to use, large volumes of antiserum are required, and such antisera are not widely available.

We report the isolation from sewage of bacteriophages that might be of use in the identification of the K1 antigen.

MATERIALS AND METHODS

Bacterial strains. *E. coli* O2:K1:H4 (strain U9/41), the standard test strain for the K1 antigen, was used for the selection and propagation of bacteriophage. A variant of this strain that lacked the K1 antigen was used as a control strain.

A total of 224 strains of *E. coli,* which had been included in a previous study (1), were used to test the specificity of bacteriophages. The strains belonged to a large number of different O:H serotypes. In addition, the standard test strains for *E. coli* antigens K2 to K99 were used as control strains.

The strains were streaked on blood agar, and a single colony was selected and retested for the presence of the K1 antigen, using the antiserum-agar technique (6). In this test the selected colonies were stab inoculated into a medium consisting of 10% meningococcus group B antiserum and 1.5% agarose in Trypticase soy broth contained in plastic petri dishes. The tests were examined after 18 h of incubation at 37°C followed by 24 h at 4°C; the appearance of a precipitin "halo" indicated the presence of K1.

Isolation of bacteriophage. A sample of settled sewage water was filtered (0.45-μm, pore size; Millipore Corp.), and 1.0 ml of filtrate was enriched in 10 ml of a nutrient broth culture of *E. coli* O2:K1:H4 (U9/41).

The resulting preparation was titrated by the soft-agar-layer technique, using *E. coli* O2:K1:H4 (U9/41) and its K1-negative variant as indicators. The resulting titer with *E. coli* O2:K1:H4 was 10^7 plaque-forming units/ml, whereas with its K1-negative variant no plaques were seen even when 0.1 ml of undiluted phage preparation had been used.

Five plaques of varying sizes and morphology were selected from a single soft-agar-layer plate and used for the preparation of five separate bacteriophage stocks, using the soft-agar-layer technique with *E. coli* O2:K1:H4 (U9/41) as the propagating strain. The resulting preparations all had titers of between 0.5×10^8 and 7.5×10^8 plaque-forming units/ml, using strain U9/41 as indicator.

Tests for specificity of bacteriophages. The five bacteriophage preparations were diluted 1/100 in nutrient broth for use in specificity testing. All five

preparations were tested by the application of 0.02-ml drops onto nutrient agar plates seeded with the single-colony subcultures of the 224 *E. coli* strains. They were similarly tested with the standard *E. coli* K antigen strains.

Immunoelectrophoresis. Certain strains were tested by immunoelectrophoresis (4), using equine meningococcus group B antiserum.

RESULTS

Antiserum-agar diffusion test. Single-colony preparations from 101 of the 224 *E. coli* strains were K1 positive and 123 were K1 negative when retested using the antiserum-agar test.

Bacteriophage tests. Of the 101 single-colony preparations that were K1 positive using the antiserum-agar technique, 93 were lysed by one or more of the bacteriophage stocks. Of the 123 single-colony preparations that were K1 negative using the antiserum-agar technique, 8 were lysed by one or more bacteriophage stocks (Table 1). None of the standard test strains for *E. coli* K antigens 2 to 99 were lysed by any of the bacteriophages.

Immunoelectrophoresis. The eight single-colony preparations that were K1 negative according to the antiserum-agar test but were lysed by one or more bacteriophage stocks were further examined by immunoelectrophoresis, using equine meningococcus group B antiserum. Five of the eight strains gave a precipitin line corresponding in position to that found using *E. coli* O2:K1:H4 (strain U9/41).

TABLE 1. *Bacteriophage sensitivity of 224 E. coli strains compared with the antiserum-agar test*

Bacteriophage sensitivity	No. of strains (%)	
	K1+	K1−
Resistant to all	8(8)	115 (93.5)
Sensitive to:		
One	9	2
Two	4	0
Three	2	1
Four	7	2
Five	71	3
Total sensitive	93(92)	8[a] (6.5)

[a] Five of these were shown to possess K1 by immunoelectrophoresis.

DISCUSSION

Bacteriophages are widely used as a diagnostic tool in bacteriology and provide a convenient method of differentiating otherwise similar organisms. Bacteriophages specific for certain surface factors of *E. coli* have been isolated from sewage, well-known examples being those specific for the F+ and Hfr mating types (3). In the present study we have isolated five bacteriophages that appear to be specific for the K1 antigen.

When single-colony isolates of *E. coli* were tested simultaneously using the antiserum-agar diffusion test and the bacteriophage isolates, good correlation was found. Only 8 of the 101 single-colony preparations that were K1 positive according to the antiserum-agar technique were resistant to all bacteriophages. Further studies may lead to the isolation of K1-specific bacteriophages with the ability to lyse these strains. Eight of the 123 single-colony preparations that were K1 negative according to the antiserum-agar technique were nevertheless lysed by one or more bacteriophages, but five of these eight were shown to possess the K1 antigen by immunoelectrophoresis. The bacteriophage test may therefore prove to be more sensitive than the antiserum-agar technique.

It is of interest that all the bacteriophages isolated after enrichment of the sewage sample with *E. coli* O2:K1:H4 were K1 specific. It is possible that the K1 antigen effectively masks all underlying structures and prevents the adsorption of bacteriophages with other specificities. It may be equally easy to isolate bacteriophages specific for other important K antigens.

Previous studies of the epidemiology of the K1 antigen of *E. coli* have used immunodiffusion techniques, which depend on the use of large volumes of specific antisera. In the present study five different K1-specific bacteriophages were used individually to detect K1. It is possible that this technique might be streamlined by pooling the individual bacteriophages to form a single test preparation. The use of K1-specific bacteriophages offers an inexpensive and convenient method for the identification of this clinically important antigen.

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