Isolation of an Lc-Specific *Escherichia coli* Bacteriophage

JOE A. FRALICK,^{1*} DANA L. DIEDRICH,² AND SHARON CASEY-WOOD¹

¹Department of Microbiology, Texas Tech University Health Sciences Center, Lubbock, Texas 79430,
and Department of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center,
New Orleans, Louisiana 70119-2799²

Received 17 August 1989/Accepted 19 December 1989

We isolated an OmpF-specific bacteriophage whose host range mutant, SQ108h2, requires the presence of the Lc porin for its attachment and which can be used to screen or select for Lc-defective mutants among *Escherichia coli* K-12 strains lysogenic for the PA-2 converting phage.

The outer membrane of gram-negative bacteria functions as a diffusion barrier to both hydrophobic and hydrophilic molecules (6, 7). This barrier is bridged by thousands of small water-filled pores composed of a few species of proteins called porins (5, 6). In *Escherichia coli* K-12 strains, OmpF and OmpC are the predominant porins and may constitute more than 50% of the total outer membrane protein mass (5). These transmembrane proteins have been extensively studied, and the genetics of their regulation are beginning to be understood (3, 5, 7). In PA-2 lysogens of K-12 strains, a genetically distinct porin, the Lc (lysogenic conversion) protein, which is specified by the *lc* gene on the temperate phage, can, under the appropriate physiological conditions, almost entirely supplant OmpC and OmpF (2, 4, 8, 9). The Lc, OmpC, and OmpF proteins are structurally related (1) and coordinately controlled (4, 9); any increase in the expression of Lc causes a concomitant decrease in the amount of OmpC and/or OmpF. These observations pose some interesting questions concerning the regulatory relationship(s) of *lc*, *ompC*, and *ompF*. Unfortunately, the genetics of Lc expression have been refractory to analysis because there has been no convenient method for selecting Lc-defective mutants. In this communication we report the isolation of a bacteriophage that utilizes the Lc protein for attachment and that can be used in the isolation of Lc-defective mutants of *E. coli* K-12 strains.

Isolation of porin-specific bacteriophages. Samples of the effluent of the Lubbock, Tex., sewage disposal plant were collected, clarified by centrifugation, and sterilized with chloroform. To enrich for porin-requiring bacteriophages, we differentially adsorbed the sewer phages to *E. coli* K-12 strains lacking one of the three porins OmpC, OmpF, and Lc and then removed the adsorbed phages and bacteria by centrifugation. The bacteriophages remaining in the supernatant were then plated on an isogenic *E. coli* strain which expressed all three porins. Plaques from these lawns were then replica plated onto lawns of the porin-deficient *E. coli* strains, and those phages which could grow on the porin-proficient host but not on one of the porin-deficient mutants were saved and further analyzed. From this search we isolated an OmpC-requiring (SQ101) and an OmpF-requiring (SQ108) bacteriophage. No Lc-requiring bacteriophage was found by this strategy. Both SQ101 and SQ108 appeared to be virulent, producing clear plaques of moderate size. No evidence of lysogeny was observed.

Isolation of host range mutants that require the Lc porin. Because OmpF, OmpC, and Lc porins are similar in structure (1), we reasoned that one might find a host range mutant of SQ101 and/or SQ108 which could use the Lc porin as a receptor. To obtain such a host range mutant, we subjected both SQ101 and SQ108 to UV irradiation and then plated them on an OmpF⁻ OmpC⁻ Lc⁺ strain of *E. coli*. Using this strategy, we were able to isolate a host range mutant of SQ108 that required either OmpF or Lc for its propagation. We designated this bacteriophage SQ108h1. By propagating SQ108h1 on an OmpF⁻ OmpC⁻ Lc⁺ strain for several rounds of replication (five consecutive lysates) and preadsorbing the phage to an OmpF⁺ OmpC⁺ Lc⁻ strain, we were able to isolate a second host range derivative, SQ108h2, that was specific in its requirement for the Lc porin for its propagation (Table 1).

Porin-specific phage sensitivity and adsorption studies. The sensitivities of a variety of genetically defined porin-deficient *E. coli* K-12 strains to our porin-specific bacteriophages were determined (Table 1). From these results it is evident that SQ101, SQ108, SQ108h1, and SQ108h2 require the OmpC, OmpF, OmpF or Lc, and Lc porins, respectively, for their propagation. That this requirement is for attachment became apparent from the phage adsorption studies presented in Table 2. The *E. coli* K-12 strains used in this study were grown exponentially in Luria broth (LB) at 37°C to a cell density of approximately 4×10^8 . They were then harvested by centrifugation and resuspended to an optical density of 1.0 (at 550 nm) in fresh LB plus chloramphenicol (100 µg/ml) to prevent phage propagation. The bacterio-

TABLE 1. Porin-specific phage sensitivity analysis

Strain	Relevant phenotype ^a			Phage sensitivity ^b			
	OmpC	OmpF	Lc	SQ101	SQ108	SQ108h1	SQ108h2
CS137	+	+	+	S	S	S	S
CS138	+	+	-	S	S	S	R
MH513	+	-	-	S	R	R	R
CS139	-	+	-	R	S	S	R
CS140	-	+	+	R	S	S	S
MH225	-	+	-	R	S	S	R
CS197	-	-	-	R	R	R	R
CS228	-	-	+	R	R	S	S

^a Phenotype with respect to presence (+) or absence (-) of the porins was determined by SDS-urea-polyacrylamide gel electrophoresis analysis.

^b Phage sensitivity was determined by cross-streaking the *E. coli* K-12 strain against the indicated bacteriophage. S, Sensitivity; R, resistance.

* Corresponding author.

TABLE 2. Adsorption analysis of porin-specific phages

Strain	Relevant phenotype ^a			Phage adsorption (%) ^b			
	OmpC	OmpF	Lc	SQ101	SQ108	SQ108h1	SQ108h2
CS137	+	+	+	23	46	90	96
CS197	—	—	—	3	<1	<1	<1
CS228	—	—	+	6	<1	93	97
CS139	—	+	—	2	90	90	2
MH513	+	—	—	89	<1	<1	<1

^a Relevant phenotype with respect to the presence (+) or absence (—) of the porins was determined by SDS-urea-polyacrylamide gel electrophoresis analysis.

^b Percent adsorption was determined from the unadsorbed phage as follows: percent adsorption = [(total phage added — unadsorbed phage)/total phage added] × 100.

phage were then added at a multiplicity of infection of approximately 0.01 and incubated at 37°C for 20 min. The number of unadsorbed phage was determined by pelleting the *E. coli* cells and determining the titers in the supernatant.

Characterization of *E. coli* K-12 mutants resistant to porin-specific phages. To determine if our porin-specific bacteriophages could be used to select for OmpC-, OmpF-, or Lc-defective mutants, we isolated *E. coli* K-12 mutants resistant to each of these phages. The protocol was to add SQ101, SQ108, SQ108h1, or SQ108h2 to cultures of CS137 (a PA-2 lysogen) at a multiplicity of infection of approximately 5 and then to plate 0.1 ml of each culture on an LB plate and incubate the plates for 24 to 48 h at 37°C. A single colony was picked from each plate and further purified on LB plates. The resistance of these colonies to porin-specific bacteriophages was determined by cross-streak analysis. Representatives of these phage-resistant mutants were grown in LB medium at 37°C, and their Triton-insoluble outer membrane proteins (2) were analyzed by sodium dodecyl sulfate (SDS)-urea-polyacrylamide gel electrophoresis (4). A representative gel of mutants resistant to porin-specific phages is presented in Fig. 1, and the corresponding bacteriophage sensitivities are given in Table 3. Note that in each case (Fig. 1, lanes 3 to 10) mutants in which the receptor protein for the selective bacteriophage was dramatically decreased or missing were isolated. In some cases more than one porin was affected (Fig. 1, lanes 5, 6, 7, 9, and 10). Whether these

FIG. 1. SDS-urea-polyacrylamide gel electrophoresis of Triton-insoluble outer membrane proteins from mutants resistant to porin-specific phages (lanes 3 to 10). C, OmpC; F, OmpF; LC, Lc; A, OmpA. Lanes: 1, CS137, lysogenic for PA-2 (OmpC⁺ OmpF⁺ Lc⁺); 2, CS138, nonlysogenic for PA-2 (OmpC⁺ OmpF⁺ Lc⁺); 3, SQ101-resistant mutant of CS137; 4, SQ108-resistant mutant of CS137; 5, SQ108h1-resistant mutant of CS137; 6 to 10, SQ108h2-resistant mutants of CS137.

TABLE 3. Phage sensitivity of mutants resistant to porin-specific phages

Strain/phage-resistant derivative	Gel lane ^a	Phage sensitivity ^b			
		SQ101	SQ108	SQ108h1	SQ108h2
CS137	1	S	S	S	S
CS138	2	S	S	S	R
CS137/SQ101 ^r -17	3	R	S	S	S
CS137/SQ108 ^r -1	4	S	R	S	S
CS137/SQ108h1 ^r -5	5	S	R	R	R
CS137/SQ108h2 ^r -7	6	S	R	R	R
CS137/SQ108h2 ^r -31	7	S	S	S	R
CS137/SQ108h2 ^r -118	8	S	S	S	R
CS137/SQ108h2 ^r -11	9	R	R	R	R
CS137/SQ108h2 ^r -811	10	R	R	R	R

^a Lane of the SDS-urea polyacrylamide gel in Fig. 1.

^b Phage sensitivity was determined by cross-streaking the *E. coli* K-12 strains and their phage-resistant mutants against the indicated bacteriophage. S, Sensitivity; R, resistance.

mutations map at a single locus remains to be determined; however, it seems unlikely that they represent multiple mutations, since no mutagenic agent was employed in their isolation. In one mutant (SQ108h2^r-7) the pleiotrophic effect can be attributed to a defect in the synthesis of the core region of the lipopolysaccharide (unpublished results).

Summary. We have isolated a host range mutant of an OmpF porin-specific phage, SQ108h2, which can be used for the selection and genetic analysis of mutants defective in the expression of the PA-2 phage-encoded Lc porin. To demonstrate its utility we isolated SQ108h2-resistant derivatives of a PA-2 (*lc*⁺) lysogen. SDS-polyacrylamide gel analysis demonstrated that the Lc protein is either missing or dramatically decreased in the outer membrane of these *E. coli* mutants. The observation that bacteriophage SQ108h1 can utilize either OmpF or Lc for its attachment suggests that these two porins may present a similar receptor sequence at the cell surface.

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