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Coliphage Which Requires Either the LamB Protein or the OmpC Protein for Adsorption to *Escherichia coli* K-12

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Either of two different proteins in the outer membrane of *Escherichia coli* K-12 (LamB and OmpC) can function in the constitution of receptor activity for a newly isolated T-even bacteriophage. This bacteriophage (SSI) differs from other T-even phages which use the OmpC protein as their receptors. The simple procedure used to isolate phage SSI may be suitable for the detection of bacteriophages with novel outer membrane receptor requirements.

Our understanding of the genetics and physiology of the proteins in the outer membranes of gram-negative bacteria such as *Escherichia coli* has been greatly assisted by the availability of a range of bacteriophages which use these proteins to adsorb to sensitive cells (13). However, a large proportion of the approximately 30 proteins in the *E. coli* outer membrane remain unstudied, in part because there are as yet no available bacteriophages which are specific for these proteins. We therefore decided to isolate novel bacteriophages (from a variety of sewage, fecal, and water samples) which might use these proteins as receptors. For this purpose, we used *E. coli* CS162 which was derived from strain P435 (Wrm Hep$^-$ [*rfa*]; devoid of all lipopolysaccharide core sugars other than ketodeoxyoctonic acid [4]) by successively selecting mutants which were resistant to bacteriophages λ, K3, T6, BF23, and T5. Outer membranes prepared from strain CS162 were shown by sodium dodecyl sulfate gel electrophoresis (10) to be devoid of the proteins which serve as receptors for these phage (LamB, OmpA, Tsx, BtuB, and FhuA [TonA]). They also contained considerably reduced amounts of the major outer membrane pore proteins OmpF (protein 1a/Ia/b/O9) and OmpC (protein 1b/Ib/c/O8), a feature which is characteristic of other *E. coli* K-12 strains with altered lipopolysaccharide structures (15). Strain CS162 had a generation time of 150 min in rich medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, pH 7.2) compared with 33 min for the Wrm$^+$ parent strain (P400 [4]). Comparisons of bacteriophage plaque counts for a sample of sewage plated on strains CS162 and P400 indicated that over 95% of the coliphage present were unable to grow on strain CS162.

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Preliminary screening of the bacteriophages isolated by using strain CS162 indicated that the isolates were of a variety of different types. Several of the bacteriophages formed plaques only on *E. coli* K-12 mutants which produced altered lipopolysaccharide or on *E. coli* B strains. These phages presumably recognize outer membrane components which are either masked or inaccessible in *E. coli* K-12 strains. Bacteriophages which formed plaques on wild-type *E. coli* K-12 were used to select bacteriophage-resistant mutants. Mutants resistant to two of the novel bacteriophages (SS1 and SS4) were shown by sodium dodecyl sulfate gel electrophoresis (10) to be devoid of the outer membrane OmpC protein and were resistant to other bacteriophages which are known to use this protein in the constitution of their receptor (Mel, TuIb, and PA-2 [9, 13]). Mutants resistant to bacteriophage SS1 were also devoid of the maltose-inducible outer membrane LamB protein (13) and resistant to bacteriophage λ. Two possible explanations for the latter phenomenon were initially considered. (i) The receptor for phage SS1 was an outer membrane component which, when removed by mutation, resulted in the inability to synthesize OmpC and LamB or in their incorrect assembly in the outer membrane (18). (ii) Bacteriophage SS1 could use either OmpC or LamB in the constitution of its receptor, and, hence, only mutants lacking both proteins would be SS1 resistant. We confirmed that the latter interpretation was correct from the following data (Table 1).

(i) Spontaneous SS1-resistant mutants appeared at a frequency of 10^{-8} to 10^{-9} in wild-type *E. coli* K-12 strains. Over 80% of the isolates studied were unable to ferment maltose in maltose EMB plates, and the Mal$^-$ λ^r phenotype was shown by P1 phage transduction experiments (7) to be cotransduced with the *aroB*$^+$

TABLE 1. *Titration of LamB- and OmpC-specific bacteriophages on various E. coli K-12 derivatives*[a]

Strain (temp [°C])	Plaque titer/ml			
	SS1	λ (V)	TuIb	SS4
W3110	2.0×10^{10}	3.4×10^{11}	2.6×10^{10}	3.4×10^{11}
W3110 *malT*::Tn*5*	3.6×10^{10}	<10	1.8×10^{10}	2.9×10^{11}
W3110 *ompC*::Tn*5*	1.4×10^{10}	3.8×10^{11}	<10	<10
W3110 *malT*::Tn*5 ompC*	<10	<10	2.0×10^{3}	<10
W3110 SS1^r *lamB ompC*	<10	<10	1.6×10^{3}	<10
pop1050	<10	<10	5.6×10^{10}	NT[b]
pop1050 *φ80supF*	4.6×10^{10}	2.0×10^{11}	7.2×10^{10}	NT
pop1050 SS1^r *ompC*	<10	<10	1.6×10^{2}	NT
pop1050 *ompC φ80supE*	5.1×10^{10}	3.2×10^{11}	7.4×10^{2}	NT
CS445 (30)	7.1×10^{10}	8.4×10^{10}	6.3×10^{7}	3.8×10^{11}
CS445 (42)	6.5×10^{10}	6.8×10^{10}	2.6×10^{3}	3.5×10^{11}
CS445 SS1^r *lamB* (30)	6.1×10^{10}	<10	2.6×10^{7}	4.5×10^{11}
CS445 *lamB* (42)	<10	<10	2.9×10^{3}	2.8×10^{11}

[a] W3110 is prototrophic, pop1050 is *lamB200*(Am) *lacZ*(Am) *rpoB metA*, and CS445 is *supD*(Ts) *ompC*(Am) *gyrA arg trp*(Am) *lacZ*(Am) Val^r. Strain CS445 produced substantially reduced levels of OmpC at 42°C and near normal levels at 30°C. The OmpC protein produced by this strain at 30°C appears to have impaired receptor activity for TuIb and Mel but not for SS1, SS4, or PA-2. The SS1^r mutant of CS445 was selected after growth at 42°C. Plaque titrations were as described previously (9).

[b] NT, Not tested.

allele into strain CS397 (*aroB351 ompB156*) at approximately 75 min on the *E. coli* K-12 genetic map (1). In the remaining 20% of cases, the strains were Mal$^+$, and the λ^r phenotype was cotransduced with *rpoB* or *metA* (91 min). We conclude that the bacteriophage λ resistance in the mutants originally isolated as SS1 resistant was due to mutations either at *malT* or *lamB*, a regulatory gene and the structural gene for the bacteriophage λ receptor in the *E. coli* K-12 outer membrane (1, 13). SS1^r Mal$^-$ mutants were also devoid of a periplasmic protein (released by cold osmotic shock [8]) which presumably corresponded to the maltose-binding protein MalE. Resistance to bacteriophage TuIb was shown by P1 transduction to be due to independent mutations which were cotransducible with *gyrA* and *glpT* (47 min) and therefore probably identical to the structural gene for OmpC (14; Lee, Schnaitman, and Pugsley, manuscript in preparation). Resistance to bacteriophage SS1 was not cotransduced with either TuIb^r or λ^r in these experiments unless the recipients were already resistant to bacteriophages λ or TuIb, respectively.

(ii) *E. coli* K-12 strains which were already resistant to bacteriophage TuIb (OmpC$^-$) or λ (LamB$^-$) gave rise to SS1-resistant mutants at considerably higher frequencies (10^{-5} to 10^{-6}) than wild-type strains (see above). *E. coli* B strains (LamB$^-$ OmpC$^-$) were fully resistant to SS1 but became sensitive upon transduction to Mal$^+$ (λ^s), using P1 phage grown on *E. coli* K-12.

(iii) When SS1-resistant mutants of strain pop1050 [K-12 *lamB*(Am)] were selected, all of the 30 independent isolates remained Mal$^+$. When these mutants were lysogenized with phage *φ80 supF*, sensitivity to bacteriophages λ and SS1 was fully restored by supression of the *lamB* mutation (Table 1) without affecting sensitivity to phage TuIb. Similar results were obtained when strain CS445 [*supD*(Ts) *ompC*(Am)] was mutated to SS1 resistance at 42°C and then tested for bacteriophage sensitivity at 30°C; sensitivity to TuIb, SS1, PA-2, Mel, and SS4 was partially or fully restored at 30°C by supression of the *ompC* mutation, whereas sensitivity to bacteriophage λ was unaffected by the temperature shift (Table 1).

(iv) Triton X-100–insoluble walls (outer membranes [2, 20]) of SS1^r mutants were unable to neutralize bacteriophage SS1. The assay system was as used by Bassford et al. (2) except that the final concentration of phage was 10^3 PFU/ml, and the entire incubation mixture was plated to count residual viable phage. In a typical assay, no neutralization was obtained with 500 μg of outer membrane prepared from SS1-resistant mutants. SS1-neutralizing activity was restored to wild-type levels (99% neutralization by ≤100 μg of outer membrane) when the strains from which the membrane were prepared were transduced to SS1 sensitivity at either the *ompC* or *malT* and *lamB* loci.

When phage SS1 was examined by electron microscopy after negative staining with phosphotungstic acid, a morphology indistinguishable from phages T4 and T6 was observed. SS1 was also morphologically similar to phages TuIb (11) and Mel (16), but different from PA-2, λ (lambdoid [12]), and SS4 (T5-like). When high-

TABLE 2. *Titration of OmpC-specific bacteriophages on E. coli K-12 strains which produce altered forms of OmpC and are devoid of LamB (malT::Tn5)*[a]

Phage	Plaque titer/ml						
	ompC$^+$	*ompC173*	*ompC174*	*ompC175*	*ompC176*	*ompC177*	*ompC178*
SS1	2.8×10^{10}	<10	1.3×10^{10}	<10	2.1×10^{10}	<10	<10
TuIb	5.3×10^{10}	1.3×10^{7}	3.8×10^{7}	3.7×10^{7}	1.2×10^{5}	5.3×10^{7}	3.8×10^{7}
T4	2.9×10^{10}	1.7×10^{9}	3.1×10^{9}	1.5×10^{9}	1.7×10^{9}	1.9×10^{9}	2.1×10^{9}

[a] T4 plaques formed on OmpC$^+$ strains were clear, and those formed on strains carrying mutant *ompC* alleles were turbid. OmpC proteins formed by strains carrying the mutant *ompC* alleles were present in normal (wild-type) amounts, but differed from wild-type OmpC protein in electrophoretic mobility during sodium dodecyl sulfate gel electrophoresis and in peptide profiles obtained by protease or cyanogen bromide cleavage (Lee et al. manuscript in preparation). The host range specificity of phage Mel was similar to that shown for TuIb.

titer stocks of phages SS1, TuIb, Mel, and T4 were dissolved in sodium dodecyl sulfate sample buffer and examined by sodium dodecyl sulfate gel electrophoresis as described by Laemmli (6), the polypeptide profiles observed were almost identical, again indicating the overall similarity of these four bacteriophages.

We then wished to determine whether the host range specificity of SS1 was similar to that of other T-even phages which use OmpC in the constitution of their receptor (TuIb and Mel) or to phage T4, which is dependent upon the presence of OmpC to maintain the correct receptor (lipopolysaccharide) configuration for adsorption to *E. coli* K-12 (5). In a previous study (Lee et al., manuscript in preparation) we used one of these bacteriophages (TuIb) to isolate *E. coli* K-12 mutants producing altered forms of the OmpC protein. The bacteriophage sensitivity patterns of these mutants were reexamined after introduction of the *malT*::Tn*5* (λ resistance) (3) allele by P1 phage transduction (Table 2). These results indicated that the host range specificity of SS1 for OmpC was different from that of TuIb, T4, Mel, SS4, and PA-2 (data not shown).

Strains CS791 (*ompC*::Tn*5*) and BZB1096 (*malT*::Tn*5*) were mutagenized with ethane methane sulfonate (7; Lee et al., manuscript in preparation) and infected with phage SS1. Survivors were screened for sensitivity to bacteriophages λ (strain CS791), TuIb, PA-2, Mel, SS4, and T4 (strain BZB1096). In the former case, all of the 30 independent Mal$^+$ mutants tested were fully resistant to bacteriophages λ and SS1. In the latter case, some of the strains tested were only partially resistant to SS1 (efficiency of plating, 10^{-2}) and were also partially resistant to phage TuIb, but remained fully sensitive to Mel, SS4, PA-2, and T4. All of the other mutants of this strain were highly resistant to all of these bacteriophages except T4 (efficiency of plating, 10^{-2} to 10^{-3}).

There was a striking resemblance between the results described here and those described previously for phage TP1 by Wandersman and Schwartz (17). This phage is a mutant of phage TuIa and can use either the OmpF protein (the receptor for TuIa) or the LamB protein as its receptor. Single-step mutants with reduced sensitivity to TP1 were isolated by Wandersman and Schwartz (17). These mutants are phenotypically and genetically indistinguishable from PerA mutants described by Wanner et al. (18) and M. Schwartz (personal communication). PerA mutants are easily distinguished from other membrane mutants by the fact that they are insensitive to a wide range of colicins (A, E2, E3, G, H, Ia, Ib, K, L, M, S4, V, and X) and are also partially resistant to bacteriophages λ, Mel, TuIa, TuIb, and SS1 (plating efficiencies, 10^{-2} to 10^{-4}) after growth in tryptone-yeast extract medium (data not shown). Similar mutants might be expected to arise among SS1-resistant derivatives of wild-type *E. coli* K-12. We therefore screened 100 independent, spontaneous, ethane methane sulfonate- and transposon Tn*5*-generated SS1-resistant mutants for changes in colicin and TuIa sensitivities. All of the mutants remained fully sensitive to these agents, presumably because the residual SS1 sensitivity in PerA-like mutants was sufficient to prevent their appearance as SS1-resistant clones.

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ADDENDUM IN PROOF

Two recent publications (C. Wandersman, F. Moreno, and M. Schwartz, J. Bacteriol. **143**:1374–1383, 1980; F. Moreno and C. Wandersman, J. Bacteriol. **144**:1182–1185, 1980) provide further characterization of PerA-like mutants and describe further derivatives

of bacteriophage TuIa with properties similar to those of SS1.

LITERATURE CITED

1. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1-56.
2. **Bassford, P. J., D. L. Diedrich, C. A. Schnaitman, and P. Reeves.** 1977. Outer membrane of *Escherichia coli*. VI. Protein alteration in bacteriophage-resistant mutants. J. Bacteriol. **131**:608-622.
3. **Berg, D. E.** 1977. Insertion and excision of the transposable kanamycin resistance determinant Tn*5*, p. 205-212. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. **Hancock, R. E. W., and P. Reeves.** 1976. Lipopolysaccharide-deficient, bacteriophage-resistant mutants of *Escherichia coli* K-12. J. Bacteriol. **127**:98-108.
5. **Henning, U., and K. Jann.** 1979. Two component nature of bacteriophage T4 receptor activity in *Escherichia coli* K-12. J. Bacteriol. **137**:664-666.
6. **Laemmli, U. K.** 1970. Cleavage of structural components during the assembly of the head of bacteriophage T4. Nature (London) **227**:680-685.
7. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Nossal, N. G., and L. A. Heppel.** 1966. The release of periplasmic enzymes by osmotic shock from *Escherichia coli* in exponential phase. J. Biol. Chem. **241**: 3055-3062.
9. **Pugsley, A. P., and C. A. Schnaitman.** 1978. Outer membrane proteins of *Escherichia coli*. VII. Evidence that bacteriophage-directed protein 2 functions as a pore. J. Bacteriol. **133**:1181-1189.
10. **Pugsley, A. P., and C. A. Schnaitman.** 1979. Factors affecting the electrophoretic mobility of the major outer membrane proteins of *Escherichia coli* in polyacrylamide gels. Biochim. Biophys. Acta **581**:163-178.
11. **Schmitges, C. J., and U. Henning.** 1976. The major proteins of the *Escherichia coli* cell envelope membrane. Eur. J. Biochem. **63**:47-52.
12. **Schnaitman, C. A., D. Smith, and M. Forn de Salsas.** 1975. Temperate bacteriophage which causes the production of a new major outer membrane protein by *Escherichia coli*. J. Virol. **15**:1121-1130.
13. **Schwartz, M.** 1980. Interaction of phages with their receptor proteins. *In* L. Randall and L. Philipson (ed.), Bacterial viruses, receptors and recognition, series B, vol. 6, virus receptors, part 1. Chapman and Hall, London.
14. **van Alphen, L., B. Lugtenberg, R. van Boxtel, A.-M. Huch, C. Verhoef, and L. Havekes.** 1979. *meoA* is the structural gene for major outer membrane protein c of *Escherichia coli* K12. Mol. Gen. Genet. **169**:147-155.
15. **van Alphen, W., B. Lugtenberg, and W. Berendson.** 1976. Heptose-deficient mutants of *Escherichia coli* K12 deficient in up to three major outer membrane proteins. Mol. Gen. Genet. **147**:263-269.
16. **Verhoef, C., P. J. de Graaf, and E. J. J. Lugtenberg.** 1977. Mapping of a gene for a major outer membrane protein in *Escherichia coli* K-12 with the aid of a newly isolated bacteriophage. Mol. Gen. Genet. **150**:103-105.
17. **Wandersman, C., and M. Schwartz.** 1978. Proteins Ia and the *lamB* protein can replace each other in the constitution of an active receptor for the same coliphage. Proc. Natl. Acad. Sci. U.S.A. **75**:5636-5639.
18. **Wanner, B. L., A. Sarthy, and J. Beckwith.** 1979. *Escherichia coli* pleiotropic mutant that reduces amounts of several periplasmic and outer membrane proteins. J. Bacteriol. **140**:229-239.