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bor Gene of Phage λ, Involved in Serum Resistance, Encodes a Widely Conserved Outer Membrane Lipoprotein

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***bor* is one of two recently identified genes of phage λ which are expressed during lysogeny and whose products display homology to bacterial virulence proteins. *bor* is closely related to the *iss* locus of plasmid ColV,I-K94, which promotes bacterial resistance to serum complement killing in vitro and virulence in animals. *bor* has a similar in vitro effect. We show here that the *bor* gene product is a lipoprotein located in the *Escherichia coli* outer membrane. We also find that antigenically related proteins are expressed by lysogens of a number of other lambdoid coliphage, in cells carrying the cloned *iss* gene, and in several clinical isolates of *E. coli*. These results demonstrate that *bor* sequences are widespread and present a starting point for mechanistic analysis of *bor*-mediated serum resistance.**

Bacteriophage λ has long served as a model system for the study of fundamental biological processes, yet even now significant parts of λ biology remain poorly understood. One area of notable obscurity is the function of the so-called accessory sequences (16) which comprise roughly a third of the λ genome and are dispensable for growth and viability under laboratory conditions. These sequences contain numerous open reading frames of unknown function, and their retention in the face of presumably long-standing selective pressures suggests that they have functions which may provide consequential selective benefits.

We have previously characterized two λ genes, *lom* and *bor*, whose distinctive features offer a novel perspective on the function of these accessory sequences and on the evolutionary significance of lysogeny by temperate phage generally (2). Both *lom* and *bor* are dispensable for phage vegetative growth, are expressed in *Escherichia coli* lysogens, and are related to bacterial virulence proteins. Lom is homologous to three such proteins, Ail, PagC, and Rck, which are involved in invasiveness of *Yersinia enterocolitica*, survival in macrophage phagosomes, and serum resistance of *Salmonella typhimurium*, respectively (23, 35, 46). A fifth member of the family, the *Enterobacter cloacae* protein OmpX, has not been associated with virulence properties (53, 54). None of these proteins is phage encoded.

The phage λ *bor* gene is closely related to the *iss* locus of the conjugative plasmid ColV,I-K94. Sequencing (13) has shown that *iss* displays a discrete block of 796 bp of DNA homology to λ covering the region from bp 46186 to 46982 in the λ sequence (50), which is at the 3′ end of R_Z, at the extreme right end of the λ genome. On either side of this region, homology to λ is not detectable. The overall DNA identity between λ and *iss* is 81%, excluding gaps. The homologous region includes *bor*, 230 bp of 5′ flanking DNA, and 274 bp of 3′ flanking material which includes the coding region for the last 79 residues of R_Z. Thus, *iss* appears to be a fragment of λ (or, less likely, vice versa), and it seems probable that the locus originated in a fairly recent recombination event between ColV,I-K94 and λ or a related phage. The *iss* open reading frame homologous to *bor* is 93% identical at the DNA level and is predicted to encode a protein of 102 amino acids 79% identical to Bor.

iss is a well-studied virulence locus. It promotes an approximately 20-fold increase in survival of *E. coli* K-12 in animal serum in vitro and an approximately 100-fold increase in animal virulence of a non-K12 *E. coli* strain (3, 13, 14). We have shown that *bor* has the same in vitro effect as *iss*, promoting an approximately 20-fold increase in survival in animal serum on *E. coli* K-12 λ lysogens (2). This finding demonstrates that the closely related structures of these proteins underlie a functional similarity. It also raises some interesting questions. For instance, are these phenotypes selectively important in the ecology of λ and *E. coli* λ lysogens? Are they important clinically? Do other lambdoid phage carry similar genes? By what mechanism do Bor and Iss produce resistance to serum complement killing? One can imagine that the selective benefits provided by *bor* in wild-type environments could be substantial for both the phage and its lysogenic host. One might expect such a locus to be strongly selected for and hence widespread. The work described here begins to address these issues.

MATERIALS AND METHODS

Strains and phage. The strains used are shown in Table 1. The transducing phage λ16-25 and its *bor*-Tn*phoA* fusion derivatives λ11.9 and λ11.10 have been described previously (2, 33). Phage 21, 82, and 424 and were a gift of Ethan Signer. The lambdoid phage 35s, 41, 43, 44, 60, 74, 83, 731, and 802 were a gift of T. Iijima (28). Media and bacteriological techniques were as described previously (34).

Bor peptide antibodies. Antiserum to the Bor protein was generated against a 12-residue C-terminal peptide, YTPLEARVYCSQ. Synthetic peptide, from Multiple Peptide Systems, Inc., was conjugated to two different carrier proteins, bovine serum albumin and keyhole limpet hemocyanin. Conjugations were performed as described previously (15), and peptide-carrier conjugates were used as immunogens. Immunizations and bleeds of rabbits were by Pocono Rabbit Farm, Inc. Sera were diluted and assayed for specific Bor reactivity by Western blotting (immunoblotting). Preliminary experiments (not shown) indicated that antisera detected a 10- to 11-kDa protein in λ lysogens of AB1157 but not in nonlysogens or strains carrying *bor*::Tn*phoA* fusions.

Western blotting. Immunodetection of nonlabeled proteins in whole-cell lysates was performed after transfer to nitrocellulose membranes (21). Typically 10 μg of whole-cell protein was separated on a sodium dodecyl sulfate (SDS)–10 to 15% polyacrylamide gel, which was then electroblotted onto a nitrocellulose filter (0.2-μm pore size; Schleicher & Schuell) in transfer buffer containing 25 mM Tris HCl (pH 8.3), 0.1% SDS, 0.2 M glycine, and 20% methanol for 12 to 18 h at 4°C at 4-V constant voltage. Filters were washed briefly with H_2O, and nonspecific binding sites were blocked by incubation in a 3% solution of bovine serum albumin (fraction 5; Sigma) in phosphate-buffered saline (PBS; 0.15 M NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4 [pH 7.5]) at room temperature for 30 to 60 min. Filters were then incubated with primary antisera

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TABLE 1. Strains used

Strain	Description	Source or reference
AB1157	*ara argE* Δ(*gpt-pro*A)*62 galK hisG kdgK lacY1 leuB6 mgl51 mtl-1* Δ*qsr'* Δ*rac rfbD1 rpsL supE thi-1 thr-1 tsx xyl-5*	B. Bachmann
CC118	*araD139* Δ(*ara-leu*)*7697* Δ*lacX74 phoA*Δ*20 galE galK thi rpsE rpoB argE*(Am) *recA1*	C. Manoil
JJ185	CC118/p185 (subclone of *bor*::Tn*phoA* 11.9 in pZ150)	This study
JJ186	CC118/p186 (subclone of *bor*::Tn*phoA* 11.10 in pZ150)	This study
JJ213	AB1157 (λ$^+$)	2
JJ214	AB1157 (λ16-25)	2
JJ215	AB1157 (λ11.9)	2
JJ216	AB1157 (λ11.10)	2
KH1071	KH933, a clinical isolate of serotype O78:K80, carrying *Bam*HIA subclone of *iss* in pBR322	M. Binns (3)
KS303	*galE galK* Δ*lacX74 rpsL* Δ*phoAPvu*II Δ*lpp5508*	K. Strauch (56)
XPh5	*lac phoR rpsL*	Laboratory collection
ZK53–ZK79	Clinical isolates of *E. coli* (see text)	R. Kolter
SL2824	Wild-type *S. choleraesuis*	B. A. D. Stocker
G2111 and G348	Isolates of *S. choleraesuis* converting phage ϕ14	B. A. D. Stocker

diluted in blotting buffer (50 mM Tris [pH 7.4], 0.15 M NaCl, 5 mM EDTA, 0.5% Tween 20, 0.25% gelatin) to an extent determined by titration experiments. Incubations were for at least 60 min at room temperature with mild agitation. Filters were then washed four times with blotting buffer and incubated with a secondary detection antibody diluted 1:7,500 in blotting buffer for at least 60 min at room temperature with mild agitation. The detection system used was alkaline phosphatase-conjugated goat anti-rabbit antibody (or rabbit anti-mouse in certain experiments; kit from Promega). Filters were then washed twice in blotting buffer and twice in alkaline phosphatase buffer (0.1 M Tris HCl [pH 9.5], 0.1 M NaCl, 5 mM $MgCl_2$), after which they were incubated in a detection solution containing 330 mM nitroblue tetrazolium and 165 mM 5-bromo-4-chloro-3-indolyl phosphate in alkaline phosphatase buffer. Color reactions were developed for various lengths of time and stopped by washing filters in H_2O.

Cellular fractionation. Isolation of inner and outer membranes was performed by differential solubilization in sodium lauryl sarcosinate (Sarkosyl) (6, 20). Cells were grown overnight in NZ medium. A 1.5-ml aliquot of culture was pelleted, washed once with PBS, resuspended in an equal volume of 100 mM Tris (pH 8.0)–0.5 M sucrose–0.5 mM EDTA–67 μg of lysozyme per ml, and incubated on ice for 20 min. Cells were pelleted, and the supernatant was collected, precipitated with 10% trichloroacetic acid (TCA), and saved as the periplasmic fraction. The pellet was resuspended in 750 ml of cold H_2O and passed repeatedly through a 26-gauge needle. Unlysed cells were pelleted, and the supernatant fraction was added to an equal volume of 20 mM Tris (pH 8.5)–10 mM EDTA–0.4 M KCl. Envelopes were pelleted at 83,500 × *g* at 4°C for 2 h in a Beckman TL-100 benchtop ultracentrifuge, and the supernatant was TCA precipitated and saved as the cytoplasmic fraction. The envelope pellet was resuspended in 300 μl of 0.5% Sarkosyl–10 mM Tris (pH 8.0)–5 mM EDTA and incubated 30 min at room temperature. This mixture was respun at 103,000 × *g* for 2 h at 15°C, and the supernatant was TCA precipitated and saved as the inner membrane fraction. TCA pellets and the post-Sarkosyl pellet were resuspended in 100 μl of SDS sample buffer and boiled prior to loading samples for SDS-polyacrylamide gel electrophoresis analysis. Gels were stained for total protein or assayed by Western blotting as described above.

Labeling with [^{3}H]palmitic acid. Whole-cell palmitate labeling was done as described by Parsot et al. (43). Cells were grown to exponential phase in LB medium, and 1.5 ml of culture was incubated with 50 μl (50 μCi) of [9, 10 (*n*)-^{3}H]palmitic acid (1.0 mCi/ml solution in ethanol; Amersham) and grown for 2 h at 37°C with aeration; the cells were then pelleted, resuspended in electrophoresis sample buffer, and boiled. For immunoprecipitations, labeled cells were TCA precipitated and proteins were further treated as described previously (56).

RESULTS

Bor is a lipoprotein. The amino-terminal region of Bor has structural features characteristic of a cleavable signal sequence (2, 60). We have previously shown that Bor is an *E. coli* envelope protein and that its export information resides in the N-terminal 18 residues (2). A significant feature of this region is the sequence Leu-Leu-Ile-Thr-Gly-Cys from residues 12 to 17. This sequence closely resembles the Leu-Leu-Ala-Gly-Cys consensus motif found in bacterial lipoproteins (the underlined Cys is the universally conserved +1 residue of mature lipoproteins and is the site of diglyceride conjugation and N acylation; the nonconsensus residues of Bor are found in known lipoproteins) (22). To test whether this motif represents a true acylation signal, lysogenic cells were labeled with [^{3}H]palmitic acid as described above and whole-cell lysates were resolved on an SDS–15% polyacrylamide gel. This procedure labels a number of proteins present in the host strain (Fig. 1, lane 1). AB1157 lysogenic for wild-type λ or the transducing derivative λ16-25 does not show a detectable new band in the 10- to 11-kDa region, but visualization is precluded by the dense coverage of this area of the gel with labeled host material, obscuring any new features (lanes 2 and 3). To circumvent this problem, we also labeled strains expressing Bor-PhoA fusion proteins, which were expected to migrate in a more legible region of the gel. Thus, in AB1157 lysogenic for a phage carrying *phoA* fusion 11.9, a new band is seen at approximately 47 kDa, the expected molecular mass of the fusion protein (lane 4, arrow). Similarly, a lysogen of phage carrying the 11.10 *phoA* fusion produces a new band of about 50 kDa, the expected molecular mass of this fusion protein (lane 5, arrow). Both bands are detected when labeled lysates are immunoprecipitated with anti-alkaline phosphatase antibody (lanes 8 and 9; the bands are very faint but present), demonstrating that these are Bor-PhoA fusion proteins and not induced host proteins. Immunoprecipitation of a strain overproducing wild-type alkaline phosphatase with anti-alkaline phosphatase antibody does not detect a band, showing that the palmitate label is not incorporated into the alkaline phosphatase portion of the fusion proteins (lane 13). The result with fusion 11.9 is noteworthy because the Bor-PhoA fusion joint is only two amino acid residues after Cys-17, the presumptive site of acylation (2, 22). This demonstrates that the fusion protein requires only the first 18 residues of Bor to be recognized by and incorporated into the lipoprotein pathway and that sequence information in the mature portion of the molecule (beyond residue +2) is not involved in this process. This finding is consistent with work done on several other lipoproteins, which shows that sequences in the mature portion are not required for recognition by this pathway (22).

To permit detection of native acylated Bor protein, strain KS303, carrying a deletion of the *lpp* gene, was lysogenized with λ$^+$ and with λ16-25. It was expected that this strain would have significantly less interfering label in the gel region of Bor, which is predicted to be only slightly larger than the major Braun lipoprotein. Indeed, the two lysogenic strains each show a new band not present in the parent which migrates slightly more slowly than Lpp (Fig. 1, lanes 10 to 12). This band is a

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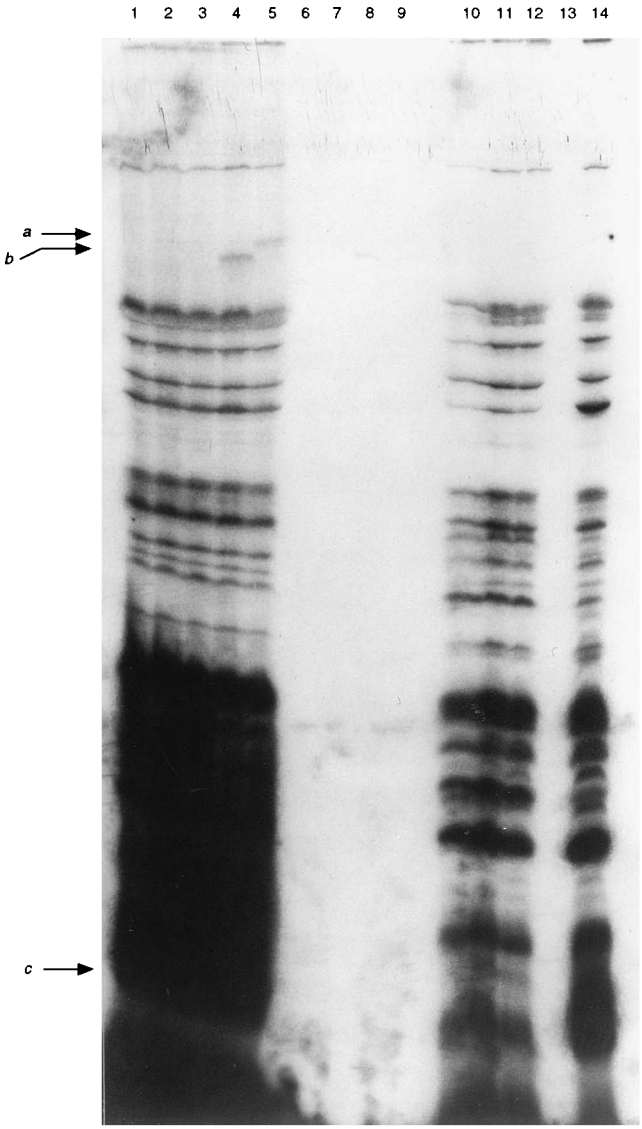

FIG. 1. Labeling of whole-cell proteins with [^{3}H]palmitic acid. Lanes: 1, AB1157; 2, AB1157(λ$^+$); 3, AB1157(λ16-25); 4, AB1157(λ11.9); 5, AB1157(λ11.10); 6, immunoprecipitation of AB1157; 7, immunoprecipitation of AB1157(λ16-25) (anti-alkaline phosphatase antibody); 8, immunoprecipitation of AB1157(λ11.9); 9, immunoprecipitation of AB1157(λ11.10); 10, KS303; 11, KS303(λ$^+$); 12, KS303(λ16-25); 13, immunoprecipitation of XPh5; 14, XPh5. Arrows: *a*, fusion protein 11.10 (lanes 5 and 9); *b*, fusion protein 11.9 (lanes 4 and 8); *c*, Bor(?) (lanes 11 and 12).

likely candidate for wild-type Bor protein. It is worth noting that Bor is one of the less abundant lipoproteins identified in this experiment. We have estimated previously that Bor is present at approximately 2,000 molecules per lysogenic cell (2).

The identity of the Bor and Iss amino acid sequences in the region of the signal sequence (13) makes it extremely likely that Iss is a lipoprotein as well.

Subcellular localization of Bor. Fractionation was performed on two lysogenic strains, one expressing wild-type Bor and the other expressing a Bor-PhoA fusion protein. JJ213 and JJ216 were subjected to Sarkosyl fractionation as described in Materials and Methods, and fractions were resolved on an SDS–15% gel which was probed by Western blotting with antisera to OmpA, Bor, and alkaline phosphatase. The final form

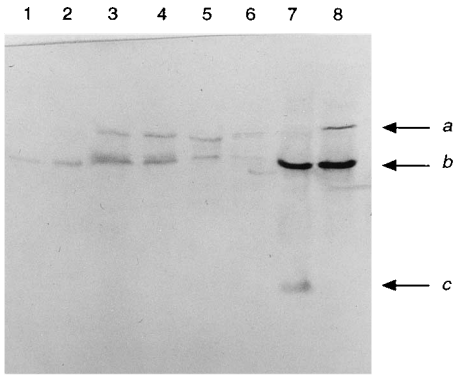

FIG. 2. Western blot showing fractionation of Bor and Bor-PhoA fusion 11.10. Lanes: 1, JJ213, cytoplasm; 2, JJ216, cytoplasm; 3, JJ213, periplasm; 4, JJ216, periplasm; 5, JJ213, inner membrane; 6, JJ216, inner membrane; 7, JJ213, outer membrane; 8, JJ216, outer membrane. Arrows: *a*, Bor-PhoA fusion protein 11-10 (lane 8); *b*, OmpA (lanes 7 and 8); *c*, Bor (lane 7).

of the blot is shown, but antisera were bound and developed sequentially to monitor band specificity.

All detectable Bor protein was found to cofractionate with OmpA, demonstrating that Bor is an outer membrane protein (Fig. 2). Furthermore, the 11.10 Bor-PhoA fusion protein showed the same fractionation pattern, which demonstrates that the first 42 residues of Bor are sufficient to target the protein to the outer membrane, analogously to what has been observed for the major Braun lipoprotein and the *excC*-encoded peptidoglycan-associated lipoprotein of *E. coli* (12, 32). Thus, Iss is likely to show the same localization as Bor.

Bor-related proteins. We screened a number of strains by Western blotting with antipeptide antibody for the presence of Bor proteins. One set of experiments involved a screen of lysogens of lambdoid phage. In a second experiment, we assayed a clinical isolate of *Salmonella choleraesuis* lysogenic for a converting phage which has been shown to promote virulence in mice and serum resistance in vitro (39). We also assayed the *E. coli* KH1071, a clinical isolate containing a subcloned fragment from ColV,I-K94 carrying *iss* (3), and a set of 10 additional clinical isolates of *E. coli.*

Figure 3 shows that the C-terminal antipeptide serum rec-

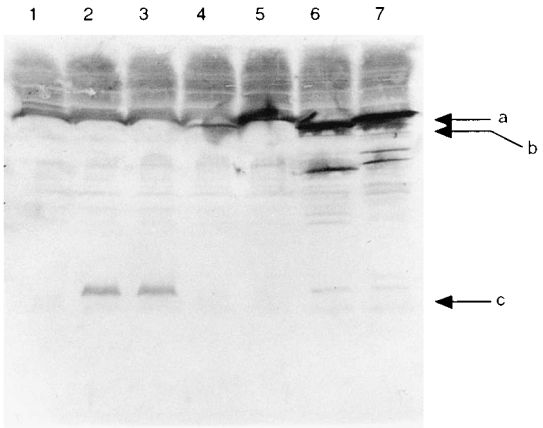

FIG. 3. Detection of Bor and Bor-PhoA fusion proteins. The filter was probed both with Bor and alkaline phosphatase antibodies (fusion proteins are seen to migrate ahead of a smear of cross-reacting material). Lanes: 1, AB1157; 2, AB1157(λ$^+$); 3, AB1157(λ16-25); 4, AB1157(λ11.9); 5, AB1157(λ11.10); 6, JJ185; 7, JJ186. Arrows: *a*, fusion protein 11.10; *b*, fusion protein 11.9; *c*, Bor.

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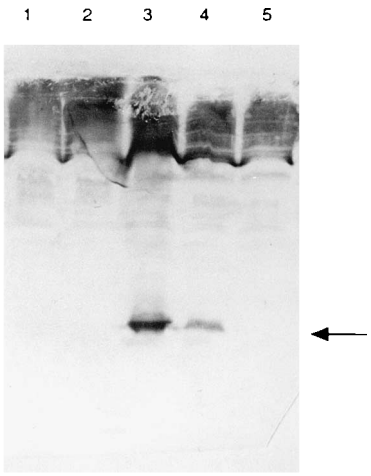

FIG. 4. ϕ80 and Iss. Lanes: 1, AB1157(ϕ80); 2, AB1157(ϕ80.2); 3, KH1071; 4, AB1157(λ$^+$); 5, AB1157.

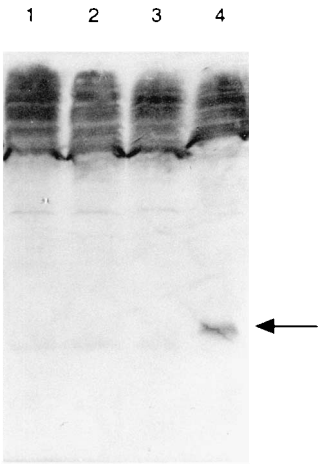

FIG. 5. *S. choleræsuis* converting phage 14. Lanes: 1, SL3824(ϕ14 G2111); 2, SL2824(ϕ14 G348); 3, SL2824; 4, AB1157(λ16-25). The arrow indicates Bor in lane 4.

ognizes Bor in lysogens of λ$^+$ and λ16-25 and that this protein is not present in lysogens of the two phage carrying *bor*::Tn*phoA* insertions 11.9 and 11.10 (lanes 4 and 5). Instead, these fusion proteins (recognized by the anti-PhoA antibody) are seen migrating at appropriate sizes in this 15% gel (arrows) and are overexpressed in strains carrying high-copy-number subclones of the fusions (lanes 6 and 7). The fusion proteins in the lysogens appear to be present at roughly the same level as the native Bor protein.

The Bor antibody strongly recognizes a protein of the same size expressed in KH1071, a non-K-12 *E. coli* strain containing a subcloned fragment carrying the *iss* gene (3) (Fig. 4, lane 3). Iss appears to be present in this strain at perhaps five times the level seen for Bor in AB1157 (lane 4). This finding represents the first evidence that the open reading frame assigned to *iss* is in fact expressed as protein, and it confirms that Iss is closely related to Bor. It should be noted that Bor and the predicted Iss protein differ at 2 of the 12 C-terminal residues recognized by the peptide antibody:

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Bor, ...YTPLEARVYCSQ
Iss, ...***R*T******
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This difference was not noted in the *iss* sequence report, in which the DNA sequence appears to have been mistranslated at these two residues (13). The changes Leu→Arg and Ala→Thr indicate some tolerance for sequence variability in this epitope in our immunodetection experiments and suggest that the antibody used here could recognize other proteins which are slightly diverged from Bor.

E. coli lysogens of a number of different phage were tested for Bor proteins by immunoblotting. Two different isolates of ϕ80 scored as negative in this assay (Fig. 4, lanes 1 and 2). Other ϕ80 isolates were also negative, as were several isolates of ϕ170 (not shown). Other negative nonlysogenic K-12 strains were W3110 λ$^-$, M0, MC4100, and CC118 (not shown).

S. choleraesuis lysogenic for serum resistance converting phage 14 was also negative under our Western blotting conditions (Fig. 5). By contrast, AB1157 lysogens of the lambdoid phage 21, 82, and 424 all scored as positive (Fig. 6). The heavier band for phage 21 was not seen reproducibly and probably represents a loading error. The lambdoid phage ϕ44, ϕ83, ϕ731, and ϕ802 also scored as positive, while several others (ϕ35s, ϕ41, ϕ43, several isolates of ϕ60, and ϕ74) were negative (Fig. 7). The faint band from ϕ83 migrates slightly more slowly than the neighboring band from ϕ802 but similarly to those from ϕ44 and ϕ731. Thus, there appears to be some variation in size among Bor proteins. These phage are not well characterized, but (except for 41, 43, and 44) have novel, non-λ immunity types (27a). ϕ44 has the same immunity as λ. This set of phage therefore extends the family in which Bor is found.

In a screen of 10 *E. coli* clinical isolates, 3 were found to express Bor-related proteins (Fig. 8). All 10 of these clinical strains were positive for colicin production (31a; colicin type was not determined). ColV plasmids are often associated with clinical *E. coli* isolates (17, 27, 37, 52) and often carry *iss* sequences (3), suggesting that *bor* or *iss* sequences in these strains may be plasmid associated. The origin and properties of the tested strains are summarized in Table 2. While this screen is of course very limited in scope, it does suggest that Bor proteins may be a common feature of *E. coli* clinical isolates.

DISCUSSION

We demonstrate in this report that the phage λ *bor* gene product is an outer membrane lipoprotein of *E. coli* lysogens.

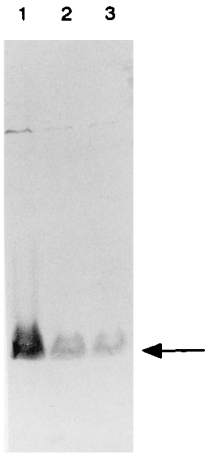

FIG. 6. Lambdoid phage. Lanes: 1, AB1157(21); 2, AB1157(82); 3, AB1157(424).

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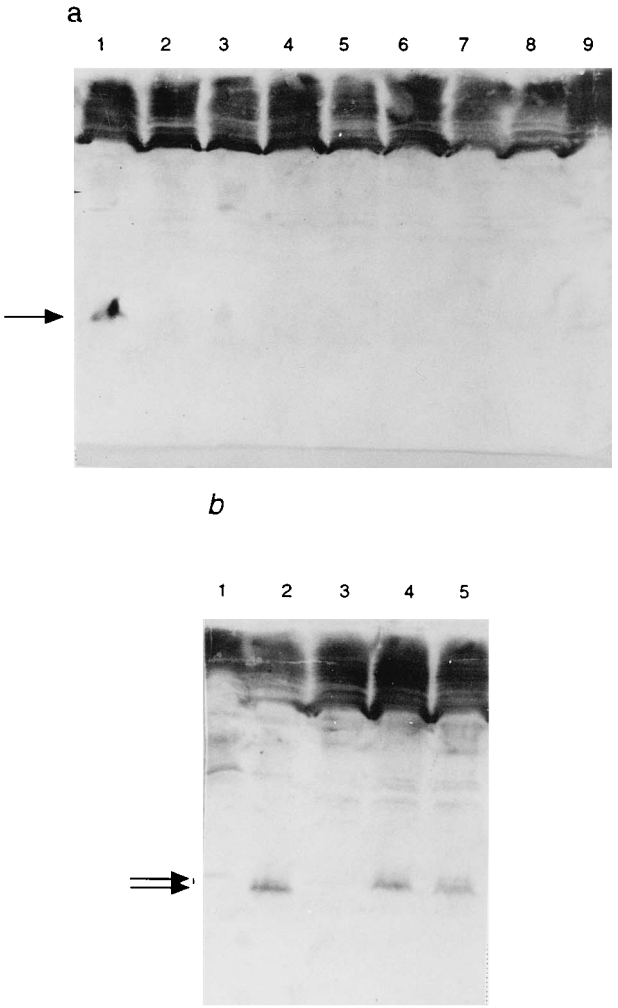

FIG. 7. (a) Lambdoid phage. Lanes: 1, AB1157(λ16-25); 2, AB1157(ϕ60S); 3, AB1157(ϕ80.2); 4, AB1157(ϕ60T); 5, AB1157(ϕ60.1); 6, AB1157(ϕ60.2); 7, AB1157(ϕ35s); 8, AB1157(ϕ41); 9, AB1157(ϕ43). (b) Lanes: 1, AB1157(ϕ83); 2, AB1157(ϕ802); 3, AB1157(ϕ74); 4, AB1157(ϕ731); 5, AB1157(ϕ44). Arrows indicate Bor proteins.

We also find that related proteins are expressed in lysogens of a number of different lambdoid coliphage and in some *E. coli* clinical isolates. Additionally, we have detected the homologous plasmid-encoded *iss* gene product.

Bor promotes resistance to serum complement killing in host bacteria, a property that it shares with the virulence protein Iss (2). The findings reported here represent a preliminary characterization of Bor, a necessary first step in understanding its mode of action. Serum resistance is a property which could play a significant role in phage and bacterial natural selection in animal hosts. It is also a hallmark of many types of pathogenic microorganisms and is one of the most extensively studied of bacterial virulence factors (7, 30, 58, 59, 62, 63). An understanding of the functioning of Bor is therefore of interest both in the context of the evolutionary functions of temperate phage accessory genes and with respect to microbial pathogenesis.

The finding that Bor is an outer membrane lipoprotein (and that Iss is very likely to be one as well, given that the two proteins are identical around the lipoprotein signal sequence motif) suggests that its effect on serum sensitivity involves

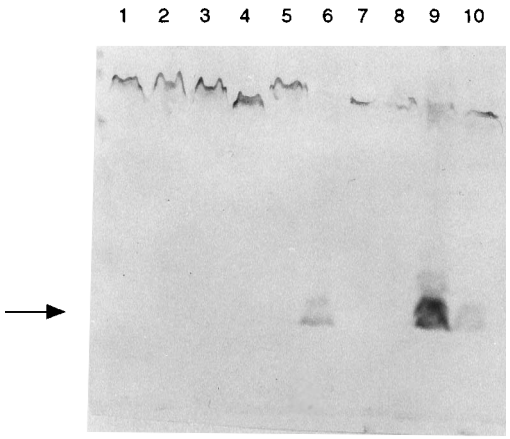

FIG. 8. *E. coli* clinical isolates. Lanes: 1, ZK53; 2, ZK54; 3, ZK55; 4, ZK56; 5, ZK57; 6, ZK58; 7, ZK59; 8, ZK68; 9, ZK71; 10, ZK79.

some change in the outer membrane. Previous mechanistic studies of Iss complement resistance are almost certainly relevant to considerations of Bor function. For instance, it has been reported (14) that *iss*-mediated protection is not seen in the presence of 0.02 M MgEGTA, an inhibitor of the classical complement pathway (40). Binns et al. (4) showed that the consumption of complement components C6 to C9 was the same in isogenic *E. coli* K-12 strains with or without Iss, suggesting that the observed protective effect was at the level of action of the C5b-9 membrane attack complex, not its formation. It is not clear what mechanism might be involved in this effect, but it is possible that Bor/Iss could act enzymatically to modify an outer membrane component. To further localize the site of action, it would be useful to know whether Bor/Iss is exposed on the cell surface.

A number of bacterial outer membrane proteins have been shown to be involved in serum resistance. These include Rck (23, 24) and Ail (5, 45), virulence proteins homologous to the λ Lom protein (which does not affect serum sensitivity itself [2]), the *E. coli* OmpA protein (61), *Streptococcus pyogenes* M protein (26), gonococcal porin protein PI (11), the related TraT proteins of the conjugative *E. coli* plasmids R100, R6-5, and ColV,I-K94 (14, 38, 41) and of *Salmonella* virulence plasmids (48, 57), and the *tpcC* gene product of *Vibrio cholerae* (42, 43). The TraT proteins and TpcC are of particular interest in the present context because they are lipoproteins (22, 43, 44). This similarity may reflect only that these proteins are all targeted to the outer membrane in this way, or it may signify

TABLE 2. Origins and Bor phenotypes of clinical *E. coli* isolates[a]

Strain	Source	Bor phenotype
ZK53	Stool	
ZK54	Stool	
ZK55	Urinary tract	
ZK56	Wound	
ZK57	Urinary tract	
ZK58	Urinary tract	+
ZK59	Urinary tract	
ZK68	Urinary tract	
ZK71	Wound	+
ZK79	Trachea	+

[a] All strains produce colicin. No correlation is apparent between the source of a strain and its *bor* status.

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some shared underlying mechanism of serum resistance. It should be noted that our estimate of Bor levels in lysogens (approximately 2,000 molecules per cell [2]), as well as the Bor (Fig. 1) and Iss levels that we find in this study (Fig. 4, lane 3; note that the amount of protein seen here is produced from a high-copy-number subclone; the level in cells carrying the large wild-type ColV,I-K94 plasmid may be somewhat lower), are roughly 5- to 10-fold lower than levels estimated for TraT proteins (38, 59). This finding, as well as the observation that the protective effect of *iss* is not dosage dependent (4, 14), suggests the possibility that Bor/Iss exerts its effect on serum sensitivity through an enzymatic rather than structural role in the outer membrane.

bor is the only phage gene known to affect serum sensitivity of a lysogenic host. The *S. choleraesuis* converting phage 14 has been shown to promote serum resistance, increased O-antigen side chain length, and mouse virulence in host bacteria, but the phage locus responsible has not yet been identified (39). We did not detect cross-reacting protein with antipeptide Bor antibody in *S. choleraesuis* lysogens (Fig. 5), but this negative result of course does not rule out the presence of a related *bor* gene in this phage. It will be interesting to characterize this particular serum resistance locus and determine whether a gene product distinct from Bor is involved. One would like to know also how widespread serum resistance loci are among temperate phage generally and whether these are related to known serum resistance genes. It is also noteworthy that eukaryotic viruses have been found to encode proteins which interfere with host complement defenses (29).

We found *bor* to be widespread among lambdoid coliphage. Related proteins were found expressed in lysogens of phage 21, 82, 424, 44, 731, 802, and 83 (Fig. 6 and 7). This finding is consistent with the notion that *bor* is a selectively advantageous locus which should be well conserved. It is also consistent with what is known about the large family of lambdoid phage. A number of DNA heteroduplex studies (19, 25, 51) have shown that these phage are constructed from a set of segments, each of which has a number of alternate forms and which can be exchanged among family members by recombination events. Thus, for instance, heteroduplex mapping has shown relatedness between phage λ, 21, 82, and 424 in the region around *bor* (25, 51), and these phage all are positive by Western blotting. By contrast, ϕ80 homology to λ in this region is punctate and hard to interpret from heteroduplex data (19). These studies also show relatedness in the *bor* region between λ and other phage.

A modular view of phage evolution considers the possibility of exchange of these phage segments with homologous sequences on other episomal elements and with "cryptic" phage remnants on the chromosome (8–10). This is significant, because *bor*/*iss* appears to be a locus which has spread in just this way: in addition to being found on the plasmid ColV,I-K94, *bor* sequences appear to be present in at least one cryptic prophage on the *E. coli* chromosome. In this instance, fine-structure restriction mapping of the *qsr′* prophage shows complete identity with the λ map in the region around *bor* (47), making it likely that those *E. coli* strains carrying *qsr′* carry chromosomal *bor* sequences as well. Furthermore, such cryptic prophage are widespread both in *E. coli* (1, 31, 55) and in other enterobacteria (49). In this context, it should also be noted that the virulence genes homologous to λ *lom* are found both on plasmids (*rck*) and on the chromosome (*pagC*, *ail*, and *ompX*), and sequences related to *rck*, *ail*, and *ompX* have been found to be widespread (23, 36, 54).

We have included in our study a screen of 10 clinical isolates of *E. coli*, of which 3 were found to express Bor proteins (Fig. 8). This small and unsystematic survey needs to be extended to a larger sample of clinical and natural isolates, but it does underscore the fact that *bor* sequences can be found in many *E. coli* strains. We note that sequences related to *iss*/*bor* have been detected in 95.5% of ColV$^+$ *E. coli* strains isolated from the blood of patients with bacteremia, from 68.8% of samples from intestinal infections, and from about 28% of all clinical strains examined, regardless of ColV production (18). These findings of course raise the question of whether *bor* sequences, and by extension λ lysogeny, are clinically important. We are aware of no studies addressing this point.

We suspect that other phage accessory genes, such as *lom*, will be found to play significant roles in the ecology of lysogenic bacteria. Our findings point to the unexpected functional importance of these accessory regions and to the close evolutionary relationship between bacteriophage and their hosts.

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REFERENCES

1. **Anilionis, A., and M. Riley.** 1980. Conservation and variation of nucleotide sequences within related bacterial genomes: *Escherichia coli* strains. J. Bacteriol. **143:**355–365.
2. **Barondess, J. J., and J. Beckwith.** 1990. A bacterial virulence determinant encoded by lysogenic coliphage λ. Nature (London) **346:**871–874.
3. **Binns, M. M., D. L. Davies, and K. G. Hardy.** 1979. Cloned fragments of the plasmid ColV,I-K94 specifying virulence and serum resistance. Nature (London) **279:**778–781.
4. **Binns, M. M., J. Mayden, and R. P. Levine.** 1982. Further characterization of complement resistance conferred on *Escherichia coli* by the plasmid genes *traT* of R100 and *iss* of ColV, I-K-94. Infect. Immun. **35:**654–659.
5. **Bliska, J. B., and S. Falkow.** 1992. Bacterial resistance to complement killing mediated by the Ail protein of *Yersinia enterocolitica*. Proc. Natl. Acad. Sci. USA **89:**3561–3565.
6. **Boyd, A., and I. B. Holland.** 1979. Regulation of the synthesis of surface protein in the cell cycle of *E. coli* B/r. Cell **18:**287–296.
7. **Brubaker, R. R.** 1985. Mechanisms of bacterial virulence. Annu. Rev. Microbiol. **39:**21–50.
8. **Campbell, A.** 1977. Defective bacteriophages and incomplete prophages, p. 259–313. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 8. Plenum Press, New York.
9. **Campbell, A.** 1988. Phage evolution and speciation, p. 1–14. *In* R. Calendar (ed.), The bacteriophages. Plenum Press, New York.
10. **Campbell, A., and D. Botstein.** 1983. Evolution of the lambdoid phages, p. 365–380. *In* R. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
11. **Carbonetti, N., V. Simnad, C. Elkins, and P. F. Sparling.** 1990. Construction of isogenic gonococci with variable porin structure: effects on susceptibility to human serum and antibiotics. Mol. Microbiol. **4:**1009–1018.
12. **Choi, D. S., H. Yamada, T. Mizuno, and S. Mizushima.** 1986. Trimeric structure and localization of the major lipoprotein in the cell surface of *Escherichia coli*. J. Biol. Chem. **261:**8953–8957.
13. **Chuba, P. J., M. A. Leon, A. Banerjee, and S. Palchaudhuri.** 1989. Cloning and DNA sequence of plasmid determinant *iss*, coding for increased serum survival and surface exclusion, which has homology with lambda DNA. Mol. Gen. Genet. **216:**287–292.
14. **Chuba, P. J., S. Palchaudhuri, and M. A. Leon.** 1986. Contributions of *traT* and *iss* genes to the serum resistance phenotype of plasmid ColV2-K94. FEMS Microbiol. Lett. **37:**135–140.
15. **Collawn, J. F., and Y. Paterson.** 1992. Production of antipeptide antibodies, p. 11.15.1–11.15.4. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. Wiley Interscience, New York.
16. **Court, D., and A. B. Oppenheim.** 1983. Phage lambda's accessory genes, p. 251–277. *In* R. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
17. **Davies, D. L., F. R. Falkiner, and K. G. Hardy.** 1981. Colicin V production by clinical isolates of *Escherichia coli*. Infect. Immun. **31:**574–579.
18. **Fernandez-Beros, M. E., V. Kissel, H. Lior, and F. C. Cabello.** 1990. Viru-

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lence-related genes in ColV plasmids of *Escherichia coli* isolated from human blood and intestines. J. Clin. Microbiol. **28:**742–746.
19. **Fiandt, M., Z. Hradecna, H. Lozeron, and W. Szybalski.** 1971. Electron micrographic mapping of deletions, insertions, inversions and homologies in the DNAs of coliphages λ and ϕ80, p. 329–354. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
20. **Filip, C., G. Fletcher, J. Wulff, and C. F. Earhart.** 1973. Solubilization of the cytoplasmic membrane of *Escherichia coli* by the ionic detergent sodium-lauryl sarcosinate. J. Bacteriol. **115:**717–722.
21. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual, p. 726. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Hayashi, S., and H. C. Wu.** 1990. Lipoproteins in bacteria. J. Bioenerg. Biomembr. **22:**451–471.
23. **Heffernan, E. J., J. Harwood, J. Fierer, and D. Guiney.** 1992. The *Salmonella typhimurium* virulence plasmid complement resistance gene *rck* is homologous to a family of virulence-related outer membrane protein genes, including *pagC* and *ail*. J. Bacteriol. **174:**84–91.
24. **Heffernan, E. J., S. Reed, J. Hackett, J. Fierer, C. Roudier, and D. Guiney.** 1992. Mechanism of resistance to complement-mediated killing of bacteria encoded by the *Salmonella typhimurium* virulence plasmid gene *rck*. J. Clin. Invest. **90:**953–964.
25. **Highton, P. J., Y. Chang, and R. J. Myers.** 1990. Evidence for the exchange of segments between genomes during the evolution of lambdoid bacteriophages. Mol. Microbiol. **4:**1329–1340.
26. **Hong, K., T. Kinoshita, J. Takeda, H. Kozono, P. Pramoonjago, Y. U. Kim, and K. Inoue.** 1990. Inhibition of the alternative C3 convertase and classical C5 convertase of complement by group A streptococcal M protein. Infect. Immun. **58:**2535–2541.
27. **Horák, V., and A. Lanc.** 1973. Colicins of *Escherichia coli* strains causing urinary tract infections, sensitivity of these strains towards colicins, and their O-serotypes. Folia Microbiol. (Prague) **18:**332–340.
27a. **Iijima, T.** Personal communication.
28. **Iijima, T.** 1963. Isolation of temperate phages from natural sources. Annu. Rep. Inst. Fermentation Osaka **1:**55–60.
29. **Isaacs, S. N., G. J. Kotwal, and B. Moss.** 1992. Vaccinia virus complement-control protein prevents antibody-dependent complement-enhanced neutralization of infectivity and contributes to virulence. Proc. Natl. Acad. Sci. USA **89:**628–632.
30. **Joiner, K. A.** 1988. Complement evasion by bacteria and parasites. Annu. Rev. Microbiol. **42:**201–230.
31. **Kaiser, K., and N. E. Murray.** 1979. Physical characterization of the "Rac prophage" in *E. coli* K-12. Mol. Gen. Genet. **175:**159–174.
31a. **Kolter, R.** Personal communication.
32. **Lazzaroni, J. C., and R. Portalier.** 1992. The *excC* gene of *Escherichia coli* K-12 encodes the peptidoglycan-associated lipoprotein (PAL). Mol. Microbiol. **6:**735–742.
33. **Lutkenhaus, J. F., H. Wolf-Watz, and W. D. Donachie.** 1980. Organization of genes in the *ftsA-envA* region of the *Escherichia coli* genetic map and identification of a new *fts* locus (*ftsZ*). J. Bacteriol. **142:**615–620.
34. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
35. **Miller, V. L., J. B. Bliska, and S. Falkow.** 1990. Nucleotide sequence of the *Yersinia enterocolitica ail* gene and characterization of the Ail protein product. J. Bacteriol. **172:**1062–1069.
36. **Miller, V. L., J. J. Farmer III, W. E. Hill, and S. Falkow.** 1989. The *ail* locus is found uniquely in *Yersinia enterocolitica* serotypes commonly associated with disease. Infect. Immun. **57:**121–131.
37. **Minshew, B. H., J. Jorgensen, M. Swanstrum, R. G. Grootes, and S. Falkow.** 1978. Some characteristics of *Escherichia coli* strains isolated from extraintestinal infections of humans. J. Infect. Dis. **137:**648–654.
38. **Moll, A., P. A. Manning, and K. N. Timmis.** 1980. Plasmid-determined resistance to serum bactericidal activity: a major outer membrane protein, the *traT* gene product, is responsible for plasmid-specified serum resistance in *Escherichia coli*. Infect. Immun. **28:**359–367.
39. **Nnalue, N. A., S. Newton, and B. A. D. Stocker.** 1990. Lysogenization of *Salmonella choleraesuis* by phage 14 increases average length of O-antigen chains, serum resistance and intraperitoneal mouse virulence. Microb. Pathog. **8:**393–402.
40. **Ogata, R. T., and R. P. Levine.** 1980. Characterization of complement resistance in *Escherichia coli* conferred by the antibiotic resistance plasmid R100. J. Immunol. **125:**1494–1498.
41. **Ogata, R. T., C. Winters, and R. P. Levine.** 1982. Nucleotide sequence analysis of the complement resistance gene from plasmid R100. J. Bacteriol. **151:**819–827.
42. **Ogierman, M. A., and P. A. Manning.** 1992. TCP pilus biosynthesis in *Vibrio cholerae* O1: gene sequence of *tcpC* encoding an outer membrane lipoprotein. FEMS Microbiol. Lett. **76:**179–184.
43. **Parsot, C., E. Taxman, and J. J. Mekalanos.** 1991. ToxR regulates the production of lipoproteins and the expression of serum resistance in *Vibrio cholerae*. Proc. Natl. Acad. Sci. USA **88:**1641–1645.
44. **Perumal, N. B., and E. G. Minkley.** 1984. The product of the F sex factor *traT* surface exclusion gene is a lipoprotein. J. Biol. Chem. **259:**5357–5360.
45. **Pierson, D. E., and S. Falkow.** 1993. The *ail* gene of *Yersinia enterocolitica* has a role in the ability of the organism to survive serum killing. Infect. Immun. **61:**1846–1852.
46. **Pulkkinen, W. S., and S. I. Miller.** 1991. A *Salmonella typhimurium* virulence protein is similar to a *Yersinia enterocolitica* invasion protein and a bacteriophage lambda outer membrane protein. J. Bacteriol. **173:**86–93.
47. **Redfield, R. J., and A. M. Campbell.** 1987. Structure of cryptic lambda prophages. J. Mol. Biol. **198:**393–404.
48. **Rhen, M., and S. Sukupolvi.** 1988. The role of the *traT* gene of the *Salmonella typhimurium* virulence plasmid for serum resistance and growth within liver macrophages. Microb. Pathog. **5:**275–285.
49. **Riley, M., and A. Anilonis.** 1980. Conservation and variation of nucleotide sequences within related bacterial genomes: enterobacteria. J. Bacteriol. **143:**366–376.
50. **Sanger, F., A. R. Coulson, G. F. Hong, D. F. Hill, and G. B. Petersen.** 1982. Nucleotide sequence of bacteriophage λ DNA. J. Mol. Biol. **162:**729–773.
51. **Simon, M. N., R. W. Davis, and N. Davidson.** 1971. Heteroduplexes of lambdoid phages: physical mapping of their base sequence relationship by electron microscopy, p. 313–328. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
52. **Smith, H. W., and M. B. Huggins.** 1976. Further observations on the association of the colicine V plasmid of *Escherichia coli* with pathogenicity and with survival in the alimentary tract. J. Gen. Microbiol. **92:**335–350.
53. **Stoorvogel, J., M. A. W. M. Van Bussel, J. Tomassen, and J. A. M. Van de Klundert.** 1991. Molecular characterization of an *Enterobacter cloacae* outer membrane protein (OmpX). J. Bacteriol. **173:**156–160.
54. **Stoorvogel, J., M. A. W. M. Van Bussel, and J. A. M. Van de Klundert.** 1991. Biological characterization of an *Enterobacter cloacae* outer membrane protein (OmpX). J. Bacteriol. **173:**161–167.
55. **Strathern, A., and I. Herskowitz.** 1975. Defective prophage in *Escherichia coli* K12 strains. Virology **67:**136–143.
56. **Strauch, K., and J. Beckwith.** 1988. An *Escherichia coli* mutation preventing degradation of abnormal periplasmic proteins. Proc. Natl. Acad. Sci. USA **85:**1576–1580.
57. **Sukupolvi, S., P. Riikonen, S. Taira, H. Saarilahti, and M. Rhen.** 1992. Plasmid-mediated serum resistance in *Salmonella enterica*. Microb. Pathog. **12:**219–225.
58. **Taylor, P. W.** 1983. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. Microbiol. Rev. **47:**46–83.
59. **Timmis, K. N., G. J. Boulnois, D. Bitter-Suermann, and F. C. Cabello.** 1985. Surface components of *Escherichia coli* that mediate resistance to the bactericidal activities of serum and phagocytes. Curr. Top. Microbiol. Immunol. **118:**197–218.
60. **von Heijne, G.** 1990. The signal peptide. J. Membr. Biol. **115:**195–201.
61. **Weiser, J. N., and E. C. Gotschlich.** 1991. Outer membrane protein A (OmpA) contributes to serum resistance and pathogenicity of *Escherichia coli* K-1. Infect. Immun. **59:**2252–2258.
62. **Wright, S. D., and R. P. Levine.** 1981. How complement kills *E. coli*. I. Location of the lethal lesion. J. Immunol. **127:**1146–1151.
63. **Wright, S. D., and R. P. Levine.** 1981. How complement kills *E. coli*. II. The apparent two-hit nature of the lethal event. J. Immunol. **127:**1152–1156.

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