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A Naturally Occurring Novel Allele of *Escherichia coli* Outer Membrane Protein A Reduces Sensitivity to Bacteriophage▽

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A novel *Escherichia coli* outer membrane protein A (OmpA) was discovered through a proteomic investigation of cell surface proteins. DNA polymorphisms were localized to regions encoding the protein's surface-exposed loops which are known phage receptor sites. Bacteriophage sensitivity testing indicated an association between bacteriophage resistance and isolates having the novel *ompA* allele.

Outer membrane protein A (OmpA) is a major, two-domain, heat-modifiable membrane protein in bacteria. The N-terminal domain is comprised of antiparallel β-strands that cross the membrane eight times, producing four large surface-exposed hydrophilic loops and three short periplasmic turns. The C terminus, located in the periplasm, is connected to the outer membrane via interactions with peptidoglycan (6). It has been proposed that OmpA is involved in the structural integrity of the outer membrane (1, 2). OmpA also acts as a phage and colicin receptor (1, 3, 7), and a number of *ompA* mutants with alterations near residues 25, 70, and 110 have been found to be resistant to bacteriophage (6, 7). The residues involved in phage resistance occur in the large surface-exposed loops of the protein, the same loops that act as phage receptors.

Outer membrane proteins similar to OmpA have been identified in 17 species of gram-negative bacteria (1). Similarities in the structure of OmpA and the high degree of similarity within the nucleotide and amino acid sequences of several enteric species indicate a high degree of evolutionary conservation. Further, a comparison of five closely related genera have shown that the β-strands are highly conserved, whereas the surface-exposed loops are highly variable (9).

An investigation to identify differences in the outer membrane proteins of *Escherichia coli* from different animal sources resulted in the identification of a novel *ompA* allele (*ompA2*). Here we describe the genetic characteristics of the novel *ompA* allele, its frequency in isolates from a range of vertebrate hosts, and an evolutionary advantage of organisms possessing the novel allele.

Outer membrane protein identification and characterization. Outer membrane proteins from three vertebrate *E. coli* isolates (H474 from a human, TA024 from a Tasmanian devil, and B194 from a varied honeyeater) were isolated using a carbonate extraction method combined with two-dimensional gel electrophoresis (8). Differential display of protein profiles from each of the three isolates showed a distinct shift in the isoelectric point of an integral outer membrane protein for isolate B194. Matrix-assisted laser desorption ionization–time of flight mass spectrometry was used to obtain mass fingerprints for each protein spot (8). Peptide analysis using appropriate databases (Profound and TrEMBL) indicated that the proteins were most similar to the *E. coli* OmpA protein.

Nucleotide analysis of the *ompA* allele from isolates characterized using proteomics revealed two sequences (*ompA1* and *ompA2*). A BLASTN search identified *ompA1* as being the *ompA* sequence of *E. coli* (GenBank accession no. OMPAECOLI). The *ompA2* allele was distinct from previously described *ompA* genes, although it was most similar (approximately 97%) to *Shigella flexneri* (GenBank accession no. AY305875). Sequencing across the variable regions of a further 14 *E. coli* isolates indicated the novel *ompA2* allele to be present in human and marsupial isolates (Table 1).

Translation and alignment of the OmpA amino acid sequences showed that the surface-exposed loops 2 and 3 were

TABLE 1. Type of *ompA* allele present in *E. coli* isolates from humans and Australian vertebrates for which sequence data were obtained

Isolate	Source	*E. coli ompA* type
H22	Human	*ompA1*
H55	Human	*ompA2*
H137	Human	*ompA1*
H312	Human	*ompA1*
H322	Human	*ompA2*
H474[a]	Human	*ompA1*
H562	Human	*ompA1*
H753	Human	*ompA1*
AH1	Human	*ompA1*
AH2	Human	*ompA1*
TA024[a]	Tasmanian devil (*Sarcophilus harrisii*)	*ompA1*
TA298	Mountain possum (*Trichosuris caninus*)	*ompA1*
TA165	Mountain possum (*Trichosuris caninus*)	*ompA1*
TA411	Brushtail possum (*Trichosuris vulpecula*)	*ompA1*
TA252	Brushtail possum (*Trichosuris vulpecula*)	*ompA1*
TA421	Eastern gray kangaroo (*Macropus giganteus*)	*ompA2*
B194[a]	Varied honeyeater (*Lichenostomus versicolor*)	*ompA2*

[a] *E. coli* isolates used for proteomic analysis.

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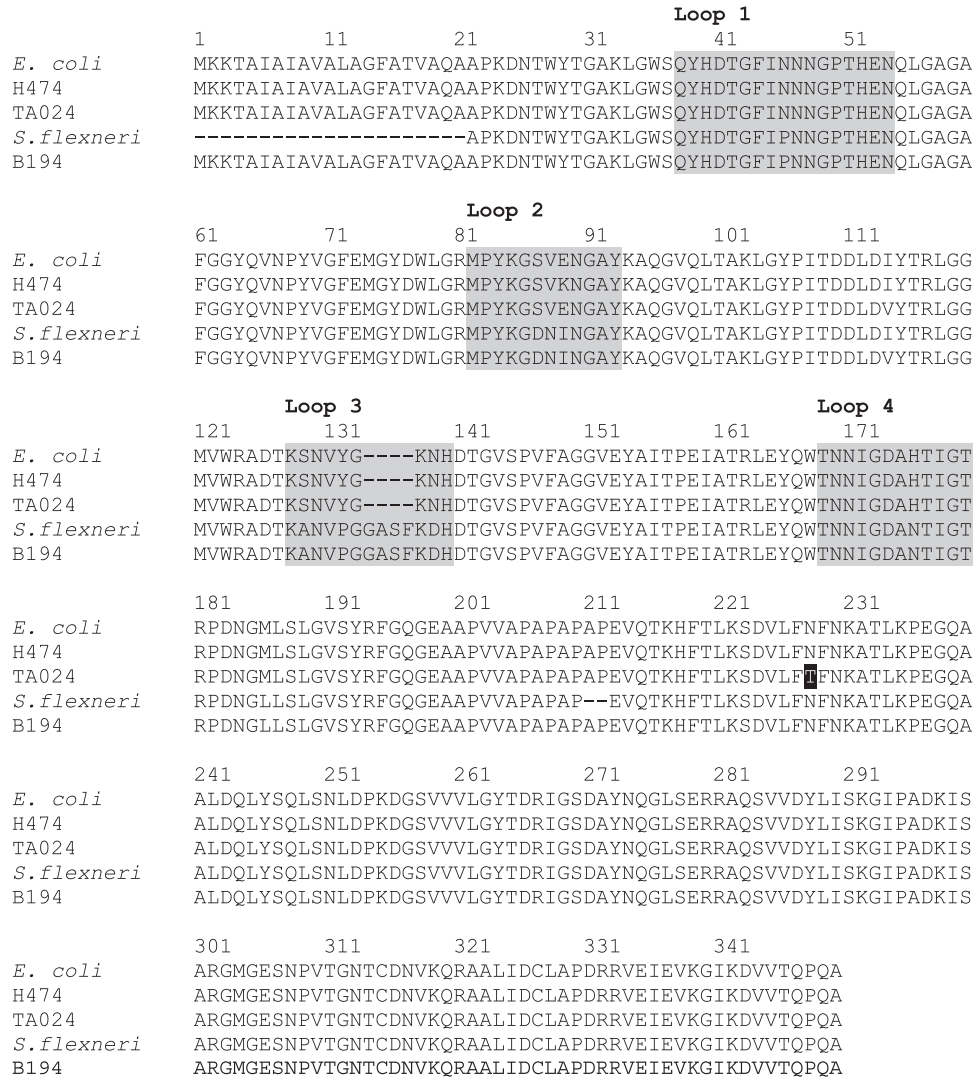

FIG. 1. Translation of the *E. coli ompA1* and *ompA2* alleles from three *E. coli* isolates H474 (human), TA024 (Tasmanian devil), and B194 (bird) and alignment to the *E. coli* and *S. flexneri ompA* sequences (GenBank accession no. ECOMPA and AY305875, respectively). Variations occur within the exposed loops of the protein (shaded gray). A single amino acid change was identified external to a loop region in isolate TA024 (shaded black).

the most variable, and single-amino-acid changes were identified in loops 1 and 4 (Fig. 1). The percentages of identity of the *E. coli ompA2* allele to the described sequences for *E. coli* and *S. flexneri* were 96.9% and 99.6%, respectively, and a single amino acid change between the *E. coli ompA2* allele and *S. flexneri* within β-strand 5 at residue 93 was observed.

Frequency of the OmpA variant in vertebrate hosts. The frequency of the *ompA2* allele was determined by screening 524 *E. coli* isolates selected from a collection of greater than 1,300 isolates sampled from a variety of sources throughout Australia. Human clinical and fecal isolates were chosen, in addition to fecal isolates from nondomesticated Australian mammals. These strains were previously screened for mitomycin C-inducible colicin production and lysogeny (4). PCR primers were designed to specifically amplify a region of the *ompA2* sequence (OmpAVF1 [5′-GGCTAACGTACCTGG TGGCGCA-3′] and OmpAVR1 [5′-CGACGATCCGGAG CCAGGCA-3′]). *E. coli* isolates were identified as having the *ompA2* allele by the presence of a 550-bp product using electrophoresis.

The novel *ompA2* allele was identified in 43% of *E. coli* strains screened using the *ompA2*-specific PCR. The frequency of the *ompA2* variant was dependent on the source of the strain (human versus animal) and the strain's *E. coli* Reference Collection (ECOR) group membership (2) (nominal logistic regression, likelihood ratio test, source, $\chi^2_1 = 2.39$, $P = 0.122$; ECOR group, $\chi^2_3 = 18.7$, $P = 0.003$; source × ECOR interaction, $\chi^2_3 = 9.9$, $P = 0.02$). The frequency of the *ompA2* allele was 45% in the human isolates and did not vary significantly among the four ECOR groups. In fecal isolates from nondomesticated mammals, the frequency was 38%, and significant differences were observed in the frequencies of the *ompA2* variant among the four ECOR groups (Fig. 2). Further, of the

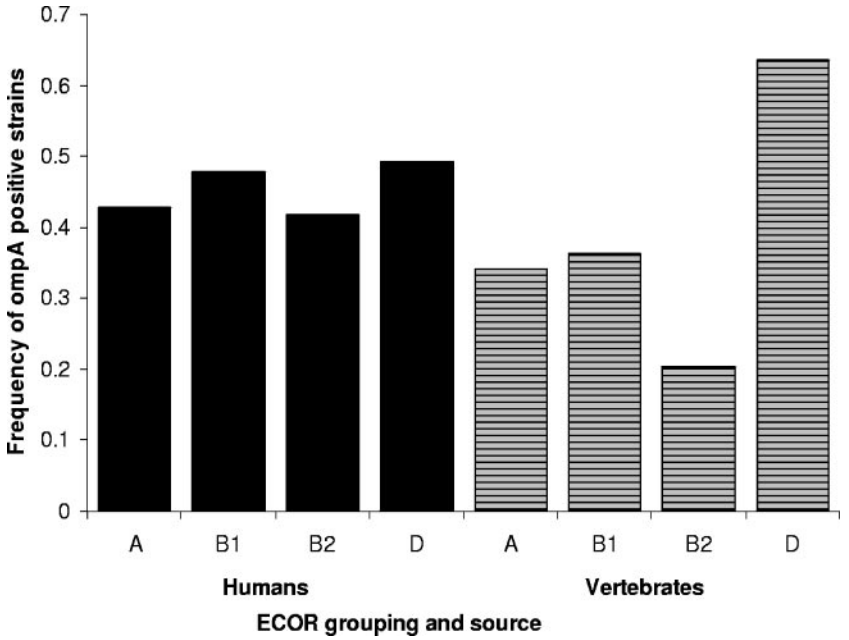

FIG. 2. Frequency of *Escherichia coli* strains carrying the *ompA2* allele with respect to source of strain and ECOR group membership of the strains.

524 strains examined, those strains with the *ompA2* allele were significantly less likely to be lysogenic (3.2%) than strains with the *ompA1* allele (16.8%) (likelihood ratio test, $\chi^2_1 = 19.1$, $P = <0.001$).

Phage sensitivity of the novel allele. We hypothesized that the high frequency of the *ompA2* allele in *E. coli* isolates conferred a selective advantage to the organism. The majority of sequence variation in the *ompA2* allele occurs within loops 2 and 3, the same regions in which laboratory-engineered mutations induced resistance to bacteriophage (6).

To determine whether the *ompA2* allele reduced sensitivity to bacteriophage, 52 *ompA1* and 52 *ompA2* isolates (13 from each of the four ECOR groups) were randomly selected (5). Lawns of the selected isolates were spotted with phage extracts (25 μl) prepared from 24 bacteriophage-positive *E. coli* strains (4). Natural *E. coli* populations having the *ompA2* allele were less sensitive to lysis by bacteriophage than strains with the *ompA1* allele were. This screening indicated that 19.2% of the *ompA*2 strains were sensitive to one or more of the 24 phages, while 39.2% of *ompA1* strains were sensitive (likelihood ratio test, $\chi^2_1 = 5.05$, $P = 0.025$). Although the phages used in this study were not identified, the use of 24 different phages from infected *E. coli* isolates would have increased the chances of screening multiple phage types.

Past studies have demonstrated that the exposed-loop regions of the protein function as phage receptors and that induced mutations in these regions alter sensitivity to bacteriophage (6, 7). We have identified a naturally occurring *ompA* allele that is associated with increased resistance to bacteriophage over typical *E. coli* OmpA expression. To confirm this hypothesis, the sensitivity of *E. coli* carrying the *ompA2* allele to bacteriophage known to use OmpA as the receptor needs to be tested. Further investigations will aid in understanding the functions of the exposed loops of the OmpA protein.

Nucleotide sequence accession numbers. Sequences from this study have been entered in GenBank under accession numbers AY682204, AY682205, and AY682206.

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