2005

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Infection and Immunity

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Infect. Immun. **2005, 73(5):2665. DOI: 10.1128/IAI.73.5.2665-2679.2005.**

INFECTION AND IMMUNITY, May 2005, p. 2665–2679 Vol. 73, No. 5
0019-9567/05/$08.00+0 doi:10.1128/IAI.73.5.2665–2679.2005

Use of In Vivo-Induced Antigen Technology for Identification of *Escherichia coli* O157:H7 Proteins Expressed during Human Infection

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Received 4 November 2004/Returned for modification 14 December 2004/Accepted 12 January 2005

Using in vivo-induced antigen technology (IVIAT), a modified immunoscreening technique that circumvents the need for animal models, we directly identified immunogenic *Escherichia coli* O157:H7 (O157) proteins expressed either specifically during human infection but not during growth under standard laboratory conditions or at significantly higher levels in vivo than in vitro. IVIAT identified 223 O157 proteins expressed during human infection, several of which were unique to this study. These in vivo-induced (ivi) proteins, encoded by ivi genes, mapped to the backbone, O islands (OIs), and pO157. Lack of in vitro expression of O157-specific ivi proteins was confirmed by proteomic analysis of a mid-exponential-phase culture of *E. coli* O157 grown in LB broth. Because ivi proteins are expressed in response to specific cues during infection and might help pathogens adapt to and counter hostile in vivo environments, those identified in this study are potential targets for drug and vaccine development. Also, such proteins may be exploited as markers of O157 infection in stool specimens.

Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 (O157) is a uniquely human pathogen that causes disease ranging from acute, self-resolving watery diarrhea to hemorrhagic colitis and the potentially fatal hemolytic-uremic syndrome (HUS). Currently, no therapies are available to lessen the potential morbidity and mortality of this infection.

E. coli O157 is thought to have evolved from a strain of enteropathogenic *E. coli* (EPEC) O55:H7 bearing the pathogenicity island termed the locus for enterocyte effacement (LEE), through the acquisition of bacteriophages encoding Shiga toxins type 1 (stx_1) and/or 2 (stx_2), acquisition of a virulence plasmid (pO157), transition of somatic antigen O55 to O157, and loss of sorbitol fermentation and β-glucuronidase activity (21). HUS as a complication of *E. coli* O157 infection has been associated with the presence of the stx_2 gene or its variant stx_{2c} in the infecting *E. coli* O157 strain (21). In addition, the characteristic attaching and effacing (A/E) lesions produced by this organism on the human colonic epithelium are a result of proteins encoded on the LEE, including the adhesion molecule intimin-γ (Eae), its receptor (Tir), the type III protein secretion system, which secretes a variety of LEE-encoded translocator proteins (EspA, EspB, and EspD) that translocate effectors into host cells, and effector proteins (Tir, EspG, EspF, Map, and EspH) that modulate the host cell cytoskeleton (21). The type III secretion system translocates Tir into the host cell, with subsequent trafficking to the host cell membrane. Intimin binding of Tir leads to host cell actin rearrangement and formation of A/E lesions. Other putative virulence factors are encoded on pO157 and include an enterohemolysin (Ehx), an immunomodulator (Lif), and a serine protease (EspP) (21). Hence several factors may be involved in *E. coli* O157 pathogenesis, and research is ongoing to understand the complexity of this infection.

The sequenced *E. coli* O157 EDL933 genome shows that although this organism shares 4.1 Mb of DNA (termed backbone) with *E. coli* K-12, it has 1.34 Mb of DNA distributed among 177 DNA segments termed O islands (OIs) that is absent in K-12 (44). Of the genes found in these OIs, only 40% have been assigned a function and several remain to be characterized (44). Collective evidence indicates that intimin-γ and the Shiga toxins act in concert with other, unidentified virulence factors, encoded by both the OI and backbone sequences, to cause the spectrum of *E. coli* O157 disease (21, 54).

To date, the main impediment to identifying a broader complement of virulence factors in this pathogen has been the lack of an animal model that mimics the spectrum of human disease. Also, the potentially fatal sequelae that can follow *E. coli* O157 infection preclude human volunteer studies. We circumvented these limitations and exploited the human immune

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response following *E. coli* O157 infection to identify a panel of microbial factors that might contribute to the pathogenicity of this organism. In particular, we used a modified immunoscreening technique called in vivo-induced antigen technology (IVIAT) (11), which enables identification of antigens expressed specifically during infection but not during growth in standard laboratory media. The rationale was that such immunogenic O157 antigens, expressed in response to unique signals encountered within the gastrointestinal tract, might contribute to pathogen adaptation and survival within the gut and hence might play important roles in the virulence of this organism. Here we report the identification of O157 proteins that are expressed during human infection. We expect the proteins identified to be potential targets for development of diagnostics, drugs, and vaccines.

MATERIALS AND METHODS

Recombinant DNA methods. Isolation of plasmid DNA, restriction digestions, and agarose gel electrophoresis were performed using standard procedures (48). All enzymes for restriction digestions, DNA modifications, and ligations were from New England Biolabs, Beverly, MA. DNA sequencing was performed at the DNA Sequencing Core Facility, Department of Molecular Biology, Massachusetts General Hospital, using ABI Prism DiTerminator cycle sequencing with AmpliTaq DNA polymerase FS and an ABI 377 DNA sequencer (Perkin-Elmer Applied Biosystems Division, Foster City, CA). Oligonucleotides for PCR and sequencing were obtained from the DNA Synthesis Core Facility, Department of Molecular Biology, Massachusetts General Hospital. Plasmids were electroporated into *E. coli* DH5α or BL21(DE3) using a Gene Pulser (Bio-Rad Laboratories, Richmond, CA) as instructed by the manufacturer. Electroporation conditions were 2,500 V at 25-mF capacitance, producing time constants of 4.8 to 4.9 ms.

Bacterial strains, plasmids, and growth conditions. An isolate of *E. coli* O157, from a patient who recovered from recent, clinically diagnosed HUS and contributed a serum sample to the pool of convalescent-phase sera for probing the expression library, was used to construct the DNA expression library (see below). *E. coli* X21-Blue(pEB313) expressed an intracellular derivative of intimin-γ, His_6–intimin-γ (35), from which the putative signal sequence of 34 amino acids had been removed (a gift of Alison O'Brien, Uniformed Services University of the Health Sciences, Bethesda, MD.). *E. coli* DH5α(pCVD468, pREP4) expressing a genetically engineered version of EspA, His_6-EspA (22), was a gift from James B. Kaper, University of Maryland School of Medicine, Baltimore, MD. Bacterial strains were grown in vitro in Luria-Bertani (LB) medium and maintained at −70°C in LB broth containing 15% glycerol. Kanamycin (Kan) and ampicillin (Amp) were used at concentrations of 50 μg/ml and 100 μg/ml, respectively.

Patient and control sera. Convalescent-phase sera (approximately 500 μl/patient) were obtained from four patients who had recovered from HUS following *E. coli* O157 infection. The ages of the patients ranged from 2 to 10 years, and sera were collected on days 13 to 96 postillness. A serum sample from a healthy pediatric patient was used as the control. All of the above serum samples were collected at the Children's Hospital and Regional Medical Center, Seattle, WA, for routine laboratory investigations, and only excess sera were used for this study. The Institutional Review Board of the Children's Hospital and Regional Medical Center approved the use of these sera, which were stored at −70°C until use.

Assessment of reactivities of pooled, unadsorbed HUS convalescent-phase and healthy control sera with immunogenic O157 proteins. Sera were assessed by examining their reactivities via colony immunoblotting (described below) against *E. coli* XL1-Blue(pEB313), expressing His_6–intimin-γ, plated onto LB-Amp plates (35) and against *E. coli* DH5α(pCVD468, pREP4), expressing His_6-EspA, plated onto LB-Amp-Kan plates (22). Both EspA and intimin-γ are expressed during human infection and targeted by the immune response (22, 35).

Adsorption of HUS convalescent-phase and control sera. To compensate for variations in immune responses of individual patients and identify the widest array of O157 antigens, equal volumes of HUS convalescent-phase serum samples from four patients were pooled and sequentially adsorbed against the *E. coli* O157 isolate recovered from one of the four patients (the same *E. coli* O157 isolate used to generate the expression library).

The adsorption protocol has been described previously (12). Briefly, a protease inhibitor cocktail formulated for bacterial cells and containing 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF; 23 mM), EDTA (100 mM), bestatin (2 mM), pepstatin A (0.3 mM), and E-64 (0.3 mM) was prepared per the manufacturer's (Sigma, St. Louis, MO) instructions and then added to intact cells and cell lysates at a dilution of 1:10. Pooled HUS convalescent-phase sera were sequentially adsorbed against in vitro-grown (LB broth, 37°C) *E. coli* O157 whole cells, cell lysates (prepared by three cycles of freezing and thawing, followed by sonication), and heat-denatured cell lysates (12). Adsorbed sera were stored at −70°C until further use.

Individual HUS convalescent-phase serum samples from each of the four patients and the control serum sample were adsorbed against whole cells, cell lysates, and heat-denatured cell lysates of the in vitro-grown (LB-Kan broth, 37°C) expression host, *E. coli* BL21(DE3) containing the native pET-30abc expression plasmids, in a similar manner.

The efficiency of adsorption of pooled HUS convalescent-phase sera was assessed using an enzyme immunoassay described previously (12) and detailed below. Adsorption efficiency was further evaluated by reacting sera with recombinant clones expressing His_6–intimin-γ and His_6-EspA via colony immunoblotting as described below.

Efficiency of adsorption of pooled HUS convalescent-phase sera. Microtiter wells were coated with 100 μl of a 1:2 dilution of a lysate of in vitro-grown *E. coli* O157 (the same isolate used to make the DNA expression library) in 50 mM carbonate buffer (pH 9.6), prepared by three cycles of freezing and thawing followed by sonication. Following overnight incubation at room temperature, wells were washed with phosphate buffered saline (PBS) containing 0.05% of Tween 20 (PBS-T) and blocked with a 1% solution of bovine serum albumin. After a 1-h incubation at 37°C, wells were emptied, and 100 μl dilutions (1:200 to 1:25,600) of sera, removed from the pool after each adsorption step, were added to wells. The wells were incubated for 1 h at 37°C and washed, after which 100 μl of a 1:1,000 dilution of horseradish peroxidase-conjugated goat anti-human affinity-purified immunoglobulin G (IgG), reactive against all classes of human immunoglobulins (ICN, Cappel, Aurora, OH), was added to the wells. Wells were incubated for 1 h at 37°C and washed with PBS-T. Reactions were developed with a 1-mg/ml solution of 2,2′-azinobis(ethylbenzthiazolinesulfonic acid) (ABTS; Sigma, St. Louis, MO) with 0.1% H_2O_2 (Sigma). The optical density at 405 nm (OD_{405}) was determined kinetically with a Vmax microplate reader (Molecular Devices Corporation, Sunnyvale, Calif.). Plates were read for 5 min at 19-s intervals, and the maximum slope for an OD change of 0.2 U was determined as milli-OD units/min (20).

Construction of an inducible *E. coli* O157:H7 genomic DNA expression library. Polymorphic amplified typing sequences, a powerful and user-friendly typing methodology for bacterial pathogens that compares well with pulsed-field gel electrophoresis (24, 25), profiled the cognate *E. coli* O157 isolates from the four HUS patients as heterogeneous. We therefore selected one isolate at random, purified genomic DNA, and generated the DNA expression library.

To generate the expression library, vector DNA was prepared by digesting with the restriction enzyme BamHI (New England Biolabs, Beverly, MA). The vectors used were the pET-30abc series of expression vectors (Novagen, Madison, WI), which permit the cloning of inserts in each of the three reading frames under the transcriptional control of the T7 phage promoter. The restriction enzyme-digested plasmid DNA was gel purified using the QIAEX II gel extraction kit (QIAGEN, Valencia, CA) and then treated with shrimp alkaline phosphatase. Genomic DNA of an *E. coli* O157 isolate from one of the four HUS patients was partially digested with the restriction enzyme Sau3AI. Following fractionation on a 1% agarose gel, DNA fragments ranging from ca. 0.5 to 1.5 kbp (insert DNA) were excised and purified using the QIAEX gel extraction kit (QIAGEN). Various ratios of insert and vector DNA were ligated and used to transform competent *E. coli* DH5α via electroporation according to standard protocols (48). Transformants were plated onto LB plates supplemented with 50 μg/ml of Kan (LB-Kan). After an overnight incubation at 37°C, growth was scraped off the plates and plasmid DNA was isolated using standard procedures (48) and used to transform electrocompetent *E. coli* BL21(DE3) (Novagen), a general-purpose expression host. To determine the percentage of transformants containing inserts, the library was plated onto LB-Kan plates, and 100 colonies were randomly picked and analyzed by colony PCR using vector-specific primers. More than 90% of transformants contained inserts ranging from 0.2 kbp to 1.8 kbp.

Screening of the expression library and identification of clones expressing immunogenic O157 proteins. The expression library was first screened with pooled unadsorbed HUS convalescent-phase sera as follows. An optimal dilution of the library in *E. coli* BL21(DE3) was plated onto LB-Kan plates to yield ca. 300 to 350 colonies per plate. After 5 h of incubation at 37°C, colonies were lifted using a nitrocellulose filter and placed, colony side up, on fresh LB-Kan plates containing 1 mM isopropyl-β-D-thiogalactoside. Plates were incubated overnight

at 30°C to induce expression of genes contained within cloned inserts. Colonies on plates were partially lysed by exposing them to chloroform vapors for 15 min in a candle jar. The filters were removed from the plates, air dried, and blocked using 5% nonfat milk in PBS (pH 7.4) for 1 h at room temperature. After a rinse with PBS-T, the filters were probed with a 1:500 dilution of pooled unadsorbed HUS convalescent-phase sera for 1 h at room temperature on a rocking platform. Filters were washed three times with PBS-T and incubated with a 1:5,000 dilution of peroxidase-labeled goat IgG directed against the human gamma globulin fraction (ICN/Cappel). Following development with an ECL chemiluminescence kit (Amersham Pharmacia Biotech), reactive clones were identified by their positions on the reference plate (the original plate from which the colonies were lifted, which was also incubated overnight at 30°C). Each reactive clone identified in the primary screen was purified further, picked with a toothpick in a grid pattern such that each test clone alternated with *E. coli* BL21(DE3)(pET-30a), the negative control, and processed as described above.

Identification of O157 proteins expressed during human infection by using IVIAT. To identify proteins expressed by *E. coli* O157 during human infection, the clones identified above were subjected to IVIAT using pooled adsorbed HUS convalescent-phase sera. Clones were picked with a toothpick onto LB-Kan plates in a grid pattern and incubated for 5 h at 37°C. Processing of filters and screening were identical to those described above, except that a 1:100 dilution of pooled adsorbed HUS convalescent-phase sera was used as a probe. Following confirmation by four additional rounds of screening, plasmid inserts were sequenced and encoded proteins were identified via BLAST against the genomic sequences of *E. coli* O157:H7 strains EDL933 and Sakai. Proteins expressed from inserts within positive clones were called in vivo-induced proteins (ivi proteins), and the genes encoding them were referred to as ivi genes. Positive clones were further probed with individual adsorbed HUS convalescent-phase serum samples from each of the four patients and the control serum sample, as described above.

Cellular localization of ivi proteins was predicted using the PSORT/PSORT-B program (http://psort.nibb.ac.jp/). Hypothetical ivi proteins were assigned putative functions using the Clusters of Orthologous Groups (COGs) database, available at http://www.ncbi.nlm.nih.gov/COG/. The online browser tool Proteome Navigator was used to compare proteins not assigned specific functions by the COGs database against Prolinks, a database of protein functional linkages derived from coevolution (2), available at http://dip.doe-mbi.ucla.edu/pronav.

Proteomic analysis of *E. coli* O157 grown in LB broth using ESIμLC-MS/MS. To determine whether ivi proteins were expressed when *E. coli* O157 was cultured under standard laboratory conditions, *E. coli* O157 grown in LB broth was subjected to microcapillary high-performance liquid chromatography combined with electrospray ionization tandem mass spectrometry (ESIμLC-MS/MS) at the Harvard Partners Center for Genetics and Genomics, Cambridge, MA. The *E. coli* O157 whole cells for one-dimensional μLC-MS/MS analysis were prepared as follows. Mid-log-phase *E. coli* O157 (OD_{600}, 0.7), cultured at 37°C in LB broth, was pelleted via centrifugation at 4°C and washed twice in deionized water. Cells were aliquoted into 1.5-ml tarred centrifuge tubes, frozen at −80°C, and lyophilized to dryness under a high vacuum. The tubes were then weighed again and the total dried cell pellet weight determined. Dried *E. coli* O157 pellets (2 mg) were dissolved in 200 μl of 6 M urea, 1% sodium dodecyl sulfate, 100 mM ammonium bicarbonate, and 10 mM dithiothreitol (pH 8.5). Samples were vortexed, and following incubation at 37°C for 1 h, 12 μl of 500 mM iodoacetamide, 100 mM ammonium bicarbonate (pH 8.5) was added to each 200-μl sample. The reaction was allowed to proceed at room temperature for 1 h in the dark. Alkylation was quenched by the addition of 2 μl of 2 M dithiothreitol in 100 mM ammonium bicarbonate, pH 8.5. Samples were then diluted eightfold with 5 mM $CaCl_2$, mixed with 20 μg of Promega sequencing-grade trypsin, and incubated at 37°C for 16 h. Following quenching with 2 μl of formic acid, samples were diluted with 2 ml of 0.1% formic acid and cleaned up using a Waters Oasis MCX cartridge. Peptides were eluted with 6% ammonium hydroxide in 50% acetonitrile, frozen, and lyophilized. Samples were redissolved in 5% acetonitrile–0.1% formic acid/water and loaded onto a 96-well plate for mass spectrometry (MS) analysis.

For MS, samples were run on an LCQ DECA XP plus Proteome X workstation from Thermo Finnigan. For each run, 85 μl of reconstituted sample was injected with a Surveyor Autosampler, while the separation was done on a 250-μm (inner diameter) by 30-cm column packed with C_{18} medium running at a 2-μl-per-minute flow rate provided from a Surveyor MS pump with a flow splitter, with a gradient of 5 to 72% water, 0.1% formic acid, and 5% acetonitrile over the course of 240 min (4 h). Two such runs were performed. Between each set of samples, two standards of a 5 Angio mix of peptides (Michrom BioResources) were run to ascertain column performance and observe any potential carryover. The LCQ was run in a top five configuration, with one MS scan and five MS/MS scans. Dynamic exclusion was set to 1, with a limit of 30 seconds. Peptide identifications were made using SEQUEST (Thermo Finnigan) through the Bioworks Browser 3.1. Sequential database searches were made using the *E. coli* O157:H7 strain EDL933 FASTA database from the European Bioinformatics Institute (http://www.ebi.ac.uk/newt/display) of the European Molecular Biology Laboratory using differential carbamidomethyl-modified cysteines and oxidized methionines. A yeast protein database was spiked in to provide noise and determine the validity of the peptide hits. In this fashion, known and theoretical protein hits can be found without compromising the statistical relevance of all the data (43). Peptide score cutoff values were chosen at cross-correlation values (Xcorr) of 1.8 for singly charged ions, 2.5 for doubly charged ions, and 3.0 for triply charged ions, along with deltaCN values of 0.1 and cross-correlation normalized values of 1. The Xcorr chosen for each peptide ensured a high confidence match for the different charge states, while the delta rank scoring preliminary cutoff ensured the uniqueness of the peptide hit; the RSP value of 1 ensured that the peptide matched the top hit in the preliminary scoring.

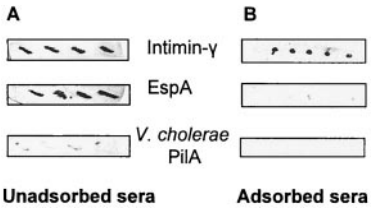

FIG. 1. Reactivities of pooled unadsorbed (A) and adsorbed (B) HUS sera against two known *E. coli* O157 virulence proteins, intimin and EspA, and an unrelated negative-control protein, PilA, from *V. cholerae*.

RESULTS AND DISCUSSION

Pooled unadsorbed HUS convalescent-phase sera reacted specifically with previously identified immunogenic O157 proteins. Pooled unadsorbed HUS convalescent-phase sera reacted strongly and specifically with *E. coli* XL1-Blue(pEB313) (35) and *E. coli* DH5α(pCVD468, pREP4) (22), expressing His_6–intimin-γ and His_6-EspA, respectively, in contrast to *E. coli* BL21(DE3) expressing recombinant *Vibrio cholerae* PilA, an irrelevant control protein (Fig. 1A). This suggested that the pool of sera possessed sufficient reactivity for probing the expression library. On the other hand, the unabsorbed healthy-control serum did not react with either of these proteins (data not shown).

Adsorption of pooled HUS convalescent-phase sera as per the IVIAT protocol resulted in selective depletion of antibodies against O157 antigens expressed in vitro. Adsorption efficiency was determined by examining the reactivities of serum aliquots from pooled HUS convalescent-phase sera after each adsorption step with lysates of in vitro-grown *E. coli* O157. There was a sharp decline in the reactivities of sera against the lysates of in vitro-grown *E. coli* O157 following the first adsorption step compared to those of the unadsorbed sera, indicating efficient depletion of antibodies against in vitro-expressed O157 proteins (Fig. 2).

Although the adsorbed sera continued to react with the clone expressing His_6–intimin-γ, it did not react with the clone expressing His_6-EspA (Fig. 1B). Since both are reportedly expressed during human infection (22, 29), as well as during in vitro growth (36), we anticipated reactivity with both might be eliminated by adsorption of the sera. We attribute the residual serum reactivity with intimin-γ to relatively weak in vitro expression of this protein, which may be insufficient to adsorb away all of the anti-intimin-γ antibodies generated in response

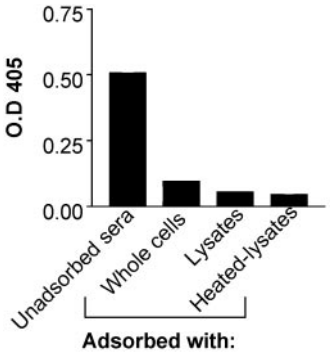

FIG. 2. Enzyme immunoassay reactivities of sera with lysates of in vitro-grown *E. coli* O157 after each step in sequential adsorption. OD_{405} values were corrected for background and for dilution during adsorption.

to significantly higher expression of this adhesin within the gastrointestinal tract.

Screening of an *E. coli* O157 genomic expression library. Primary screening of ca. 50,000 clones of an *E. coli* O157 genomic expression library, using pooled unadsorbed HUS convalescent-phase sera, yielded 918 reactive clones. IVIAT of these clones using pooled adsorbed HUS convalescent-phase sera identified 223 persistently reactive clones containing unique inserts as determined from nonredundant databases.

ivi proteins included previously identified *E. coli* O157 virulence-related proteins. IVIAT identified four proteins previously reported to have a putative role in *E. coli* O157 virulence (1) (Table 1). (i) Intimin-γ is a LEE-encoded outer membrane adhesin that acts in concert with other LEE-encoded proteins to generate the A/E lesion (21) and to effect binding to host nucleolin (50) to tether the bacterium to the enterocyte. (ii) QseA, a backbone-encoded LysR-type quorum-sensing *E. coli* transcriptional regulator, is part of the regulatory cascade that controls expression of O157 virulence factors via quorum sensing (52). QseA is also present in other gastrointestinal pathogens such as EPEC (QseA) and *V. cholerae* (AphB). Following activation by the furanone AI-2, QseA activates transcription of Ler, the positive activator of the LEE operon, and thereby influences expression of putative virulence factors from the LEE. A *qseA* mutant is impaired in the secretion of LEE-encoded proteins via the type III secretion system, also encoded on the LEE (53). (iii) TagA, a pO157-encoded inner membrane lipoprotein (41), is a protein of unknown function, but a putative role in *E. coli* O157 virulence has been suggested because of its presence in a diverse collection of *E. coli* O157 strains (41) and the fact that a homolog in *V. cholerae* is regulated by ToxR, a transcriptional regulator that governs expression of several *V. cholerae* virulence factors (13). (iv) MsbB2, a pO157-encoded inner membrane acyltransferase, facilitates the synthesis of hexaacyl lipid A, the form with maximal biological activity (23). MsbB2 reportedly functions to suppress minor modifications of lipid A. Acting in conjunction with MsbB1, another homologous acyltransferase expressed from the chromosome, MsbB2 facilitates the synthesis of lipid A of maximal biological activity, which interacts optimally with the host immune system to evoke an immune response to LPS (23). Strongly supporting a role for MsbB2 in *E. coli* O157 virulence is the fact that LPS reportedly acts synergistically with the Shiga toxins, especially Stx2, in the pathogenesis of HUS (19). Further support for a likely role in *E. coli* O157 virulence is suggested by observations that MsbB2 contributes to the virulence of related pathogens such as *Shigella flexneri* and septicemic *E. coli* O18:K1:H7 strain H16 and that it influences the expression of virulence-related surface structures in diverse pathogens (23).

ivi proteins expressed from the *E. coli* O157 backbone. A total of 181 ivi proteins of diverse functional classes were expressed from the backbone (Table 2). Those involved in biosynthesis and metabolism (51.38%) may have functions essential for bacterial growth in vivo, a feature imperative for bacterial pathogenicity. Also, consistent with the anaerobic gut environment, IVIAT identified glycolytic enzymes, hydrogenases involved in fermentation of carbon compounds, an alcohol dehydrogenase, and reductases (including two cryptic nitrate reductases) involved in energy generation from carbohydrates via anaerobic respiration and fermentation (42). These results were expected and consistent with those of other studies of diverse organisms using techniques such as transcriptional profiling (59), in vivo expression technology (15, 33), recombinase in vivo expression technology (4), signature-tagged mutagenesis (14, 37, 38), selective capture of transcribed sequences (SCOTS) (7), and IVIAT of other organisms (5).

Transport ivi proteins (18.78%) included diverse ABC-type transporters and phosphotransferase systems (PTS), permeases, transport proteins involved in the transport of diverse molecules, iron uptake proteins such as FhuA (a transporter of ferrichrome [Fe^{3+}] and a receptor for phages and colicins), CirA, an iron-regulated receptor for uptake and transport of colicin I, uncharacterized transport proteins, and, in particular, an anaerobically induced permease, TdcC, expressed from the *tdc* operon, which encodes the transport and degradation of L-threonine and L-serine. Inactivation of the activator, TdcA,

TABLE 1. ivi proteins with reported roles in O157 pathogenesis

IVIAT clone	Insert localization on EDL933 genome	Gene/protein/function[a]	Bacterial-cell localization[b]	Contig/plasmid accession no.	No. of reactive sera[c]	
					HUS sera	Control serum
H-310	OI 148	*eae*/intimin/attaching and effacing	Outer membrane	AE005595	4	0
H-13, -75	pO157	*msbB2*/MsbB/lipid A acyltransferase	Inner membrane	AF074613	4	0
H-124	pO157	L7031/probable *toxR*-regulated lipoprotein TagA	Inner membrane	AF074613	4	0
H-314	Backbone	*qseA*/transcriptional regulator, LysR type; quorum-sensing *E. coli* regulator A	Cytoplasm	AE005552	4	0

[a] Putative functions of hypothetical proteins determined from the COGs database when available.
[b] Predicted by the PSORT/PSORT-B program.
[c] Sera from four patients with HUS and one healthy person were tested individually.

TABLE 2. Backbone-encoded O157 ivi proteins

General function and IVIAT clone	Gene/protein/specific function[a]	Bacterial-cell localization[b]	Contig accession no.	No. of reactive sera[c]	
				HUS sera	Control serum
Macromolecule synthesis					
H-5, -20	*topA*/DNA topoisomerase type I, omega protein/DNA replication, repair, restriction/modification	Cytoplasm	AE005379	4	0
H-9	*dsbA*/protein disulfide isomerase I, essential for cytochrome *c* synthesis and formate-dependent reduction/protein translation and modification	Periplasm	AE005616	4	0
H-22, -23	*crcB*/putative protein involved in chromosome condensation	Inner membrane	AE005242	4	0
H-37	*purK*/phosphoribosylaminoimidazole carboxylase (AIR carboxylase), CO_2-fixing subunit/purine ribonucleotide biosynthesis	Cytoplasm	AE005233	4	0
H-40	*purD*/phosphoribosylglycinamide synthetase (GAR synthetase)/purine ribonucleotide biosynthesis	Cytoplasm	AE005632	4	0
H-42	*apbA*/thiamine biosynthesis, alternate pyrimidine biosynthesis	Inner membrane	AE005221	4	0
H-110	*recR*/DNA replication, repair, restriction/modification	Cytoplasm	AE005487	4	0
H-121	*cysD*/ATP:sulfate adenylytransferase, subunit 2	Cytoplasm	AE005502	4	0
H-145, -183	*glgP*/glycogen phosphorylase/polysaccharide modification	Cytoplasm	AE005566	4	0
H-211	*mutH*/methyl-directed mismatch repair/DNA replication, repair, restriction/modification	Cytoplasm	AE005512	4	1
H-230	Z2851/putative Rad3-related DNA helicase	Cytoplasm	AE005403	1	0
H-245, -260	*dfp*/flavoprotein affecting synthesis of DNA and pantothenate metabolism/DNA replication, repair, restriction/modification	Inner membrane	AE005591	4	0
H-313	*mutS*/methyl-directed mismatch repair/DNA replication, repair, restriction/modification	Cytoplasm	AE005501	4	0
Small-molecule synthesis					
H-11	*ggt*/gamma-glutamyltranspeptidase/biosynthesis of cofactors, carriers: thioredoxin, glutaredoxin, glutathione	Periplasm	AE005568	4	1
H-60	Z3637/putative thiamine pyrophosphate-requiring enzyme—isoleucine, leucine, valine biosynthesis	Cytoplasm	AE005468	4	0
H-71	*dapD*/2,3,4,5-tetrahydropyridine-2-carboxylate *N*-succinyl transferase/amino acid biosynthesis: lysine	Cytoplasm	AE005192	2	1
H-116, -137	*glyA*/serine hydroxymethyltransferase/amino acid biosynthesis: glycine	Inner membrane	AE005485	4	0
H-117	*accC*/acetyl-CoA carboxylase, biotin carboxylase subunit/fatty acid and phosphatidic acid biosynthesis	Cytoplasm	AE005553	4	0
H-131, -133	*hemK*/putative protoporphyrinogen oxidase/biosynthesis of cofactors: heme, porphyrin	Cytoplasm	AE005338	4	0
H-132	*alaS*/alanyl-tRNA synthetase/tRNA modification	Inner membrane	AE005498	4	0
H-164	*folE*/GTP cyclohydrase/biosynthesis of cofactors, carriers: folic acid	Cytoplasm	AE005447	4	0
H-174	*nadE*/NAD synthetase/biosynthesis of cofactors, carriers: pyridine	Cytoplasm	AE005397	4	0
H-177	*hisS*/histidine-tRNA synthetase/tRNA modification	Inner membrane	AE005480	4	1
H-194	Z2789/putative thiosulfate sulfur-transferase	Periplasm	AE005398	4	0
H-227	*cstC*/acetylornithine delta-aminotransferase/amino acid biosynthesis: arginine	Inner membrane	AE005397	4	0
H-267	*lysS*/lysine-tRNA synthetase/tRNA modification	Cytoplasm	AE005519	4	0
H-279, -280, -282, -283, -308, -312	*proB*/gamma-glutamate kinase/amino acid biosynthesis: proline	Inner membrane	AE005202	4	0
Macromolecule degradation					
H-79	*hycI*/protease/processing of C-terminal end of the large subunit of hydrogenase 3	Cytoplasm	AE005500	2	0
H-99	Z5946/putative restriction endonuclease S subunits	Cytoplasm	AE005666	2	0
H-100, -253	Z3649/putative cellulase M and related protein	Cytoplasm	AE005469	4	0
H-140, -154, -215, -218, -219, -222	*dcp*/dipeptidyl carboxypeptidase II/degradation of proteins, peptides, glycopeptides	Cytoplasm	AE005351	4	0
H-173	Z1305/putative ATP-dependent protease	Inner membrane	AE005285	2	0
H-185	*malS*/alpha-amylase/degradation of polysaccharides	Periplasm	AE005584	4	0
H-188	*uvrB*/excision nuclease subunit B/degradation of DNA	Cytoplasm	AE005259	4	0
H-236	Z2427/putative metal-dependent amidase/aminoacylase/carboxypeptidase	Cytoplasm	AE005372	4	0
H-242	*exo*/5′–3′ exonuclease/degradation of DNA	Inner membrane	AE005508	4	0
H-304, -307, -309	*endA*/endonuclease I/degradation of DNA	Periplasm	AE005525	4	0
Small-molecule degradation					
H-7	*fadA*/thiolase I/degradation of fatty acids	Cytoplasm	AE005615	4	0
H-8	*rnt*/RNase T/degrades tRNA	Cytoplasm	AE005388	4	0
H-46, -67	*xylA*/D-xylose isomerase/degradation of carbon compounds	Cytoplasm	AE005583	4	0
H-58	Z0666/putative dihydroorotase and related cyclic amidohydrolases	Cytoplasm	AE005232	4	0
H-74	*hflB*/integral membrane peptidase/degrades sigma 32	Inner membrane	AE005546	4	0

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TABLE 2—*Continued*

General function and IVIAT clone	Gene/protein/specific function[a]	Bacterial-cell localization[b]	Contig accession no.	No. of reactive sera[c]	
				HUS sera	Control serum
H-91, -93	*malY*/enzyme that blocks biosynthesis or degrades endogenous Mal inducer, probably aminotransferase/degradation of carbon compounds	Cytoplasm	AE005386	4	0
H-104	*galK*/galactokinase/degradation of carbon compounds	Inner membrane	AE005253	4	0
H-166	*celF*/phosphor-beta-glucosidase/degradation of carbon compounds	Cytoplasm	AE005396	4	0
H-175	*treF*/trehalase/degradation of carbon compounds	Inner membrane	AE005577	4	0
H-178, -241	*yciA*/putative acyl-CoA hydrolase	Cytoplasm	AE005342	2	0
H-301	*malZ*/maltodextrin glucosidase/degradation of carbon compounds	Inner membrane	AE005219	4	0
Energy/metabolism					
H-3	*yhaF*/putative 2,4,-dihydroxyhept-2-ene-1,7-dioic acid aldolase	Cytoplasm	AE005541	4	0
H-16	Z2579/putative dimethyl sulfoxide reductases	Inner membrane	AE005382	4	0
H-19	Z2779/putative arginine/ornithine *N*-succinyl transferase beta subunit	Cytoplasm	AE005397	4	0
H-29	*fba*/fructose-bisphosphate aldolase, class II/glycolysis	Cytoplasm	AE005522	4	0
H-35	Z2723/putative oxidoreductase	Cytoplasm	AE005392	4	0
H-39	*hycG*/hydrogenase/carbon fermentation	Cytoplasm	AE005500	4	0
H-49, -50, -52, -53, -134	*adhP*/alcohol dehydrogenase/anaerobic respiration	Cytoplasm	AE005357	4	0
H-54	*ycaH*/putative tetraacyldisaccharide-1-P4′-kinase	Cytoplasm	AE005281	4	1
H-72	*narW*/cryptic nitrate reductase 2, delta subunit/anaerobic respiration	Cytoplasm	AE005359	4	0
H-85	*dmsC*/dimethyl sulfoxide reductase, subunit C/anaerobic respiration	Inner membrane	AE005279	4	0
H-88	*ccmH*/possible subunit of heme lyase/electron transport	Inner membrane	AE005452	4	1
H-103, -165	*ygjL*/putative NADPH dehydrogenase	Inner membrane	AE005538	4	0
H-105	*nrfE*/formate-dependent nitrite reductases/anaerobic respiration	Inner membrane	AE005640	4	0
H-108	*phoA*/alkaline phosphatase/central intermediary metabolism	Periplasm	AE005217	4	1
H-111	*yhdJ*/putative methyltransferase	Cytoplasm	AE005554	4	0
H-114, -142	*visC*/putative 2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductases	Inner membrane	AE005521	4	0
H-118	*citF*/citrate lyase alpha chain/central intermediary metabolism	Inner membrane	AE005241	4	0
H-122	*yleB*/putative 2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductases	Inner membrane	AE005245	4	0
H-138	*citE*/citrate lyase beta chain/central intermediary metabolism	Inner membrane	AE005241	4	0
H-147	*ygfZ*/putative aminomethyltransferase related to GcvT	Cytoplasm	AE005520	4	0
H-149	*nrdB*/ribonucleoside diphosphate reductase, beta subunit, B2/central intermediary metabolism	Cytoplasm	AE005456	4	0
H-153	*agaV*/*N*-acetylgalactosamine-specific IIB component 2 (EIIB-AGA)/central intermediary metabolism	Inner membrane	AE005542	4	0
H-160, -264	Z4220/putative dehydrogenase	Inner membrane	AE005518	4	0
H-167	*nuoL*/NADH dehydrogenase I, chain L/anaerobic respiration	Inner membrane	AE005460	4	1
H-171, -306	*hybD*/probable processing element for hydrogenase-2/anaerobic respiration	Inner membrane	AE005529	4	0
H-186	*nuoM*/NADH dehydrogenase I, chain M/anaerobic respiration	Inner membrane	AE005459	4	1
H-205	Z3775/putative dehydrogenase	Outer membrane	AE005480	4	0
H-216	Z4018/putative flavodoxin	Cytoplasm	AE005499	4	0
H-217, -221, -223	Z5951/putative GTPase (G3E family)	Cytoplasm	AE005666	4	0
H-224	*yhjL*/putative oxidoreductase	Cytoplasm	AE005579	4	0
H-233, -288	*narY*/cryptic nitrate reductase 2, beta subunit/anaerobic respiration	Cytoplasm	AE005359	4	0
H-237	*solA*/putative sarcosine oxidase-like protein	Periplasm	AE005316	2	0
H-239	*yidS*/putative dehydrogenase (flavoprotein)	Cytoplasm	AE005600	4	0
H-244	*ugd*/UDP-glucose-6-dehydrogenase/central intermediary metabolism	Inner membrane	AE005428	4	0
H-254	*yliI*/putative dehydrogenase	Periplasm	AE005265	4	0
H-258	Z3401/putative oxidoreductase	Inner membrane	AE005446	4	0
H-270, -276	*epd*/D-erythrose-4-phosphate dehydrogenase/central intermediary metabolism	Cytoplasm	AE005523	4	0
H-277	*ybjT*/putative dTDP-glucose enzyme	Inner membrane	AE005268	4	0
H-287	*yraL*/putative methyltransferase	Cytoplasm	AE005543	4	0
H-292	Z3734/putative metalloprotease	Inner membrane	AE005477	4	0
H-295	*yeaA*/putative peptide methionine sulfoxide reductases	Cytoplasm	AE005401	4	0
H-300	*wecB*/UDP-*N*-acetyl glucosamine-2-epimerase/central intermediary metabolism	Cytoplasm	AE005610	4	0
H-305	*tktA*/transketolase 1 isoenzyme/central intermediary metabolism	Cytoplasm	AE005524	4	0
H-315	*pflD*/formate acetyltransferase 2/anaerobic respiration	Cytoplasm	AE005626	4	0
Regulatory					
H-1	*basS*/BasS/sensor protein for BasR, involved in macromolecule synthesis	Inner membrane	AE005644	4	0
H-14	*phoQ*/PhoQ/sensor protein; global regulation	Inner membrane	AE005328	4	0
H-24	*mopB*/GroES chaperone/folding and ushering proteins	Cytoplasm	AE005648	2	0
H-27	*pqiA*/paraquat-inducible protein A/not characterized	Inner membrane	AE005285	4	1
H-45, -87, -290, -293	*xylR*/XylR/regulates the *xyl* operon, involved in xylose utilization	Cytoplasm	AE005583	4	0
H-81, -83	*ydeW*/putative transcriptional regulator	Inner membrane	AE005353	4	0
H-82	*glnG*/GlnG/response regulator for *gln* operon, involved in glutamine biosynthesis; interacts with sensor GlnL	Cytoplasm	AE005617	4	0

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TABLE 2—*Continued*

General function and IVIAT clone	Gene/protein/specific function[a]	Bacterial-cell localization[b]	Contig accession no.	No. of reactive sera[c]	
				HUS sera	Control serum
H-86	*rcsF*/RcsF/regulates the *rcs* regulon, involved in colanic acid synthesis; interacts with *rcsB*	Outer membrane	AE005195	4	0
H-94	*yieP*/putative transcriptional regulator	Cytoplasm	AE005607	4	1
H-120, -148	*glnL*/GlnL/histidine protein kinase sensor for the GlnG regulator; glutamine biosynthesis	Inner membrane	AE0055617	4	1
H-127	*yifB*/putative 2-component regulator	Periplasm	AE005608	4	1
H-141	*uidR*/UidR/regulates the *uid* operon, involved in beta-glucuronidase synthesis	Cytoplasm	AE005385	4	0
H-159	*prpR*/PrpR/regulates the *prp* operon, involved in propionate catabolism	Cytoplasm	AE005212	4	1
H-192	*yfeR*/putative transcriptional regulator, LysR type	Cytoplasm	AE005471	4	0
H-193	*uvrY*/putative 2-component regulator	Cytoplasm	AE005414	4	0
H-195	*ybcZ*/putative 2-component sensor	Inner membrane	AE005236	4	0
H-201	*yeiL*/putative transcriptional regulator	Inner membrane	AE005448	4	0
H-256	*yifA*/putative transcriptional regulator	Cytoplasm	AE005607	4	0
H-262	*fucR*/FucR/positive regulator of the *fuc* operon	Cytoplasm	AE005508	4	0
H-274	*fliS*/FliS/regulates flagellar biosynthesis	Cytoplasm	AE005415	4	0
H-278	*hydH*/HydH/sensor kinase for HydG, hydrogenase 3 activity	Inner membrane	AE005632	4	0
H-297	*narX*/NarX/nitrate/nitrate sensor, histidine protein kinase, acts on NarL regulator	Inner membrane	AE005339	2	0
H-303	*yhjC*/putative transcriptional regulator, LysR type	Inner membrane	AE005577	4	0
H-311	Z2724/putative Arac-type regulator	Cytoplasm	AE005392	4	0
Transport					
H-4, -289	*ascF*/AscF; PTS system enzyme II/transport of small molecules: arbutin, salicin, cellobiose	Inner membrane	AE005500	4	0
H-10	*ybhS*/putative ABC-type multidrug transport system, permease component	Inner membrane	AE005260	4	1
H-12	*wzxC*/putative export protein; protein, peptide secretion	Inner membrane	AE005430	4	0
H-15	*′flhB*/export of flagellar proteins	Inner membrane	AE005410	4	0
H-28	*yohM*/putative ABC-type uncharacterized transport system, permease component	Inner membrane	AE005437	4	0
H-31	*tdcC*/TdcC; anaerobically induced permease/transport of amino acids: L-threonine, L-serine	Inner membrane	AE005540	4	0
H-34	*malF*/MalF; permease/transport of small molecules: maltose	Inner membrane	AE005636	4	0
H-44, -200, -213, -250, -251, -286	*yegT*/putative nucleoside permease	Inner membrane	AE005436	4	0
H-47, -66, -266	*ycbQ*/putative chaperone	Outer membrane	AE005284	4	0
H-59	*yedE*/putative transport system permease protein	Inner membrane	AE005415	4	0
H-73	Z4150/putative transport protein	Inner membrane	AE005512	4	0
H-76	*yhfC*/putative transport protein	Inner membrane	AE005559	4	0
H-92	*yaeM*/putative ATP-binding component of transport system	Inner membrane	AE005193	4	0
H-101	Z2654/putative chaperone distantly related to HSP70-fold metalloprotease	Cytoplasm	AE005387	4	0
H-109, -168, -169, -172, -182, -184	*livG*/LivG; ATP-binding component of high-affinity branched-chain amino acid transport system/transport of amino acids, amines	Cytoplasm	AE005569	4	0
H-115	*ugpC*/UgpC; ATP-binding component of glycerol-3-phosphate transport system/transport of small molecules: carbohydrates, organic acids, alcohols	Cytoplasm	AE005568	4	0
H-125, -275	*ycdG*/putative xanthine/uracil permease	Inner membrane	AE005300	4	0
H-126	*kefC*/KefC/transport of cations: K^+ efflux antiporter, glutathione regulated	Inner membrane	AE005181	4	0
H-143	Z3262/putative ADP-ribosylglycohydrolase	Inner membrane	AE005436	4	0
H-163	*yhfM*/putative amino acid/amine transport protein	Inner membrane	AE005560	2	0
H-176	Z5178/putative PTS component; transport of carbohydrates, organic acids, alcohols	Inner membrane	AE005600	4	0
H-187	*wzb*/putative tyrosine phosphatase	Inner membrane	AE005432	4	0
H-197	*kgtP*/KgtP; alpha-ketoglutarate permease/transport of small molecules: carbohydrates, organic acids, alcohols	Inner membrane	AE005489	4	0
H-202, -261	*fhuA*/FhuA/transport of ferrichrome (Fe^{3+}) and antibiotics, acts as a receptor for phages and colicins	Outer membrane	AE005191	4	0
H-206	*nagE*/NagE; PTS system *N*-acetylglucosamine-specific enzyme IIABC/transport of amino acids, amines	Inner membrane	AE005246	4	0
H-225	*sgaB*/putative PTS, galactitol-specific IIB component	Cytoplasm	AE005652	4	0
H-226	Z2786/putative ABC-type uncharacterized transport system, periplasmic component	Periplasm	AE005398	4	0
H-228	*yaaU*/putative transport protein	Inner membrane	AE005181	4	0
H-240	*cycA*/CycA; permease/transport of amino acids and amines: D-alanine, D-serine, glycine	Inner membrane	AE005653	4	0
H-252	*oppB*/OppB; oligopeptide transport permease protein/transport of large molecules: proteins, peptides	Inner membrane	AE005342	4	0
H-271	Z5839/putative ATP-binding component of ABC-type transport system	Inner membrane	AE005655	4	0
H-273	Z3589/putative transporting ATPase	Cytoplasm	AE005464	4	0
H-296	*araG*/AraG; ATP-binding component of high-affinity L-arabinose transport system/transport of small molecules: L-arabinose	Inner membrane	AE005411	2	1
H-316	Z2605/putative arginine/ornithine antiporter	Inner membrane	AE005384	4	0

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TABLE 2—*Continued*

General function and IVIAT clone	Gene/protein/specific function[a]	Bacterial-cell localization[b]	Contig accession no.	No. of reactive sera[c]	
				HUS sera	Control serum
Environmental adaptation (chemotaxis, motility, attachment, cell division)					
H-30	*trg*/Trg; methyl-accepting chemotaxis protein III, ribose sensor receptor/regulator; chemotaxis and motility	Inner membrane	AE005363	4	0
H-36	*osmY*/OsmY; hyperosmotically inducible protein/osmotic adaptation	Periplasm	AE005668	4	0
H-41, -130, -152, -265	*yjbB*/putative alpha-helix protein	Inner membrane	AE005634	4	0
H-55	*ftsI*/peptidoglycan synthetase/septum formation	Inner membrane	AE005185	4	0
H-62, -63, -64, -65	*mepA*/murein D,D-endopeptidase/cell envelope synthesis	Periplasm	AE005464	4	0
H-77	*sfmH*/SfmH/fimbrial assembly	Inner membrane	AE005234	4	0
H-95	*yaeH*/putative structural protein	Cytoplasm	AE005192	4	0
H-96	*cirA*/CirA/receptor for iron-regulated colicin I	Outer membrane	AE005447	4	0
H-150	*yhjD*/putative membrane protein	Inner membrane	AE005577	4	1
H-203	Z3481/putative membrane protein	Inner membrane	AE005454	4	0
H-234	*mltB*/membrane-bound lytic murein transglycosylase B/murein sacculus synthesis	Outer membrane	AE005498	3	0
H-246	*slt*/soluble lytic murein transglycosylase/murein sacculus synthesis	Inner membrane	AE005670	4	0
H-268	*tsr*/methyl-accepting chemotaxis protein I, serine sensor receptor/regulator, chemotaxis and motility	Inner membrane	AE005667	4	0
H-281	*mdaA*/MdaA/modulator of drug activity A	Cytoplasm	AE005266	4	0
H-284	*yibP*/putative membrane protein	Inner membrane	AE005588	4	0
H-302	*phoE*/PhoE/outer membrane porin protein E	Outer membrane	AE005202	4	0
Phage related					
H-209	Z1782/unknown, encoded within CP-933N	Inner membrane	AE005323	4	0
H-238	*hflX*/GTP-binding subunit of protease specific for lambda cII repressor/degradation of proteins, peptides, glycopeptides	Cytoplasm	AE005650	2	0
Unknown					
H-43, -208, -291	*yfiH*/uncharacterized conserved protein	Cytoplasm	AE005490	4	0
H-57	*ydbD*/unknown	Periplasm	AE005365	4	0
H-68, -69	*ychA*/uncharacterized conserved protein	Cytoplasm	AE005338	4	0
H-80	*yfbB*/putative enzyme	Inner membrane	AE005458	2	0
H-97	*ybfE*/unknown	Cytoplasm	AE005246	4	1
H-157	*yacC*/unknown	Cytoplasm	AE005188	4	0
H-170	Z4888/unknown	Inner membrane	AE005573	4	0
H-179	*yciI*/unknown	Inner membrane	AE005342	4	0
H-190	*yjjX*/unknown	Cytoplasm	AE005670	2	0
H-198	*yaaH*/unknown	Inner membrane	AE005177	4	0
H-212	*ybiM*/unknown	Inner membrane	AE005261	4	1
H-243	Z2619/unknown	Outer membrane	AE005385	4	0

[a] Based on homology to the sequenced *E. coli* O157 strain EDL933 genome. Putative functions of hypothetical proteins were determined from the COGs database when available.
[b] Predicted by the PSORT/PSORT-B program.
[c] Sera from four patients with HUS and one healthy person were tested individually.

results in hyperadherence of *E. coli* O157 to cultured epithelial cells due to derepression of OmpA expression (54).

Regulatory proteins (13.81%) comprised, among others, QseA, a LysR-type transcriptional activator (described above) (52), and several two-component regulatory systems that govern the virulence of diverse pathogens (17). Some of these are functionally interlinked, as evidenced by earlier reports (8, 10, 16, 58) and the Prolinks database, suggesting that IVIAT identified proteins that sense and integrate diverse environmental signals (such as anaerobiosis, cation limitation, acid, and excess, toxic levels of extracytoplasmic Fe^{3+}) and help *E. coli* O157 mount a coordinated cellular adaptive response to counter the hostile host environment. The two-component regulatory systems IVIAT identified were (i) the sensor molecule, NarX, of the NarL-NarX system, which in the absence of oxygen responds to nitrate or nitrite and acts via NarL, the response regulator that activates expression of enzymes involved in nitrate respiration and represses enzymes involved in respiration of other electron acceptors (27, 46); (ii) the sensor kinase component, PhoQ, of the PhoP-PhoQ system, which responds to extracytoplasmic levels of Mg^{2+} and Ca^{2+} (involved in the adaptation to Mg^{2+} limitation) (8) and to Zn^{2+} excess (10); (iii) the sensor component, BasS, of the BasR-BasS system, which governs the response to excess extracytoplasmic Fe^{3+} (58) and mild acid pH (51); (iv) the sensor protein, GlnL, of the GlnG-GlnL system, which responds to low ammonia concentrations and stimulates ammonia assimilation (40); and (v) HydH, the sensor for HydG, which primarily responds to high periplasmic Zn^{2+} and Pb^{2+} concentrations and nonspecifically activates the expression of hydrogenase 3, an enzyme involved

in hydrogen production during fermentation (28). IVIAT also identified an outer membrane lipoprotein, RcsF, that transduces a signal in response to glucose and zinc to the RcsC/YojN/RcsB/RcsA phosphorelay system, which in turn controls the *rcs* regulon (target genes), encoding enzymes for the colanic acid exopolysaccharide capsule (10), an acid-adaptive response that protects *E. coli* O157 from environmental stresses such as acid and heat (34). Other ivi proteins that were part of this functional group and likely to impact in vivo survival of *E. coli* O157 were (i) PrpR, a regulator of the *prp* operon involved in the catabolism of propionate, a short-chain fatty acid (SCFA) that can be detrimental to *E. coli* O157 at high concentrations (exposure to SCFA is a stress condition, and catabolism may serve to decrease the concentration of this SCFA [26, 45]); (ii) FucR, a positive regulator of the *fuc* operon encoding enzymes for metabolism of L-fucose, a component of both mucus and glycans on enterocytes (42) (following experimental inoculation of mice, *E. coli* O157 reportedly is found attached to both mucus and enterocytes [in contrast to nonpathogenic *E. coli*, which is found in mucus alone] [39] and may utilize L-fucose as one nutrient source to multiply and outcompete other flora to establish infection); (iii) FliS and FliT, which along with FliD negatively regulate the export of the anti-sigma factor, FlgM, to prevent expression of the flagellar regulon (which may promote in vivo survival, since overproduction of flagella is deleterious to bacterial growth) (61); and (iv) paraquat-inducible protein A (PqiA), an inner membrane protein of uncharacterized function.

ivi proteins functioning in environmental adaptation (8.84%) included methyl-accepting chemotaxis proteins (MCPs), a protein that was part of the adaptive response to hyperosmolarity, a colicin expressed in response to iron-limiting conditions, a modulator of drug activity, and two proteins of the PhoB regulon expressed as part of the adaptive response to phosphate limitation. Specifically, IVIAT identified two MCPs, namely, Trg, a receptor for the periplasmic ribose and galactose binding proteins, and Tsr, the serine sensor receptor, both of which are regulators of chemotaxis and motility (40). Interestingly, MCPs were also highly expressed during human infection with *V. cholerae* as identified by IVIAT (12). IVIAT also identified OsmY, a periplasmic protein of unknown function that is induced in response to hyperosmolarity (60). This protein is expressed as part of the Rcs regulon, which includes genes encoding the synthesis of the exopolysaccharide colanic acid capsule (see above) and possibly functions in the transport of an alternative osmolyte (10). One of the ivi proteins in this subgroup was CirA, an iron-regulated receptor for colicin homologous to a siderophore iron uptake system, which is also expressed under iron-limiting conditions by other pathogens such as *Salmonella enterica* serovar Typhimurium (16). Interestingly, as in previous studies (16, 58), IVIAT identified proteins expressed in response to iron limitation (FhuA and CirA), as well as those expressed in response to extracytoplasmic iron excess (BasS of the BasR-BasS two-component system; see above). Other ivi proteins included MdaA, a modulator of drug activity, and two proteins that are part of the Pho regulon and are expressed as part of the adaptive response to limiting phosphate in the environment, namely, PhoE, an outer membrane porin functioning in the transport of various anions, and a periplasmic phosphate ester hydrolase, PhoA, involved in the degradation of nontransportable organophosphates (40). The PhoB regulon is required for colonization of the rabbit small intestine by *V. cholerae* (55) and regulates *hilA* and invasion genes in *Salmonella* serovar Typhimurium (31). A possible role in *E. coli* O157 virulence is also suggested by the fact that in vivo expression of PhoB in an avian-pathogenic *E. coli* (APEC) strain during experimental infection of chickens was identified by SCOTS (7).

Phage-related proteins (1.11%) included an inner membrane protein of unknown function and a nonspecific protease that degrades the lambda repressor cII. Proteins of unknown function (6.08%) rounded off ivi proteins expressed from the backbone. Collectively, these results suggested that defined backbone ivi proteins not only support pathogenicity by facilitating in vivo survival but also regulate and indirectly complement pathogen-specific virulence factors.

ivi proteins expressed from OIs. The 37 ivi proteins expressed from OIs included 13 phage-related proteins (Table 3). Because phage proteins include both Stx1 and Stx2 in EHEC, and because they also include proteins that influence every stage of infection of mammalian hosts by diverse pathogens (56), they are potential virulence factors and warrant further evaluation. Although IVIAT did not identify either Stx1 or Stx2 (both are also produced during in vitro growth in LB broth) (47), it identified two homologous ivi proteins of unknown function, one expressed from each of the phages that encode Stx1 and Stx2. These two ivi proteins are homologous to ivi proteins expressed from several other cryptic prophages (Table 3).

Particularly interesting was the fact that certain OIs (OIs 36 and 71) containing cryptic prophages expressing ivi proteins also expressed nonphage, non-LEE (Nle) effectors (proteins encoded outside the LEE but secreted via the type III secretion apparatus encoded on the LEE) (6). The presence of Nle homologs, such as NleA and NleF (OI 71) and NleB, NleC, and NleD (OI 36), in related pathogens and the requirement of NleA for full virulence of *Citrobacter rodentium*, a pathogen of mice, suggest a probable role for phage proteins expressed from these OIs in *E. coli* O157 virulence (9).

IVIAT identified 24 clones whose inserts encoded proteins expressed from OI sequences that are not part of phage genomes (Table 3). These included intimin-γ, expressed from the LEE (OI 148); WbdP, a cytoplasmic glycosyltransferase (OI 84) involved in the synthesis of the O-polysaccharide antigen (57); WaaD, a putative periplasmic glycosyltransferase (OI 145) involved in biosynthesis of the oligosaccharide core of LPS; and numerous ivi proteins with putative or unknown functions (Table 3). The identification of enzymes involved in both O-antigen and LPS core biosynthesis by IVIAT was expected, because LPS is a broadly recognized virulence determinant of pathogenic gram-negative bacteria, and transcripts of genes encoding such enzymes were identified during infection of chickens with APEC by using SCOTS (7). Other nonphage ivi proteins that could impact *E. coli* O157 virulence included a putative arylsulfatase (OI 40) involved in the scavenging of sulfate and implicated in the ability of *E. coli* K1 to invade brain microvascular endothelial cells (18) and a putative recombinant hot spot A (RhsA) protein (OI 30) that reportedly contributes to genomic plasticity (30). Transcripts

TABLE 3. OI- and pO157-encoded O157 ivi proteins

IVIAT clone	Insert localization on EDL933 genome	Gene/protein/function[a]	Bacterial-cell localization[b]	Contig/plasmid accession no.	No. of reactive sera[c]	
					HUS sera	Control serum
Assigned function: H-285	OI 84	*wbdP*/glycosyltransferase/cell surface polysaccharides and antigen synthesis	Cytoplasmic	AE005429	4	0
Putative function						
H-6	OI 47	Z1554/putative ABC transport, lipoprotein release, permease component/cell wall biogenesis	Inner membrane	AE005305	4	0
H-17	OI 28	Z0634/putative cytoplasmic membrane, ABC-type bacteriocin/ lantibiotic exporter/defense mechanism	Inner membrane	AE005229	4	0
H-25	OI 108[d]	Z3936/putative transposase, IS*30* family	Cytoplasm	AE005493	4	0
H-33	OI 43	Z1214/predicted esterase of the alpha-beta hydrolase superfamily	Cytoplasm	AE005276	4	0
H-48, -51, -89, -90	OI 172	Z5901/putative helicase with a unique C-terminal domain including metal-binding cysteine cluster	Cytoplasm	AE005661	4	0
H-78	OI 9	Z0348/putative arabinose efflux permease/carbohydrate transport	Inner membrane	AE005205	2	0
H-84	OI 17	Z0419/putative ABC-type transport permease	Inner membrane	AE005211	4	0
H-98	OI 145	*waaD*/putative lipopolysaccharide biosynthesis enzyme (glycosyl-transferase)	Periplasm	AE005590	4	0
H-102	OI 138	Z4856/putative acyl-CoA synthetase/AMP-(fatty) acid ligase	Inner membrane	AE005571	4	0
H-113	pO157	L7004/putative hemolysin expression-modulating protein—hypothetical protein	Cytoplasm, inner membrane	AF074613	4	1
H-129	OI 157	Z5415/predicted permease	Inner membrane	AE005619	2	0
H-135	OI 30	Z0705/putative RhsA/Rhs family protein	Inner membrane	AE005236	4	0
H-136	pO157	L7091/putative nickase	Cytoplasm	AF074613	4	0
H-151	OI 40	Z1089/putative arylsufatase A	Cytoplasm	AE005267	4	1
H-231	OI 172	Z5888/predicted transcriptional regulator	Cytoplasm	AE005659	4	0
H-263	OI 139	Z4882/uncharacterized protein encoded in hypervariable junctions of pilus gene clusters	Cytoplasm	AE005572	4	0
H-298	pO157	*sopA*/putative plasmid partitioning protein A	Inner membrane	AF074613	4	0
Phage related						
H-18	OI 52	Z1930/putative hydrolase or acyltransferase (alpha/beta hydrolase family), encoded within CP-933X	Inner membrane	AE005334	4	1
H-38, -56	OI 52	Z1883/putative DNA-packaging protein encoded within CP-933X	Cytoplasm	AE005330	4	0
H-70	OI 36	Z0964/putative DNA-packaging protein encoded within CP-933K	Cytoplasm	AE005256	4	1
H-106	OI 45[d]	Z1433/unknown, encoded within BP-933W	Cytoplasm	AE005295	4	0
H-112	OI 52	Z1882/putative phage DNA-packaging protein, NU1 subunit of terminase, encoded in CP-933X	Cytoplasm	AE005330	4	0
H-128	OI 52	Z1888/putative capsid protein of prophage CP-933X	Cytoplasm	AE005330	4	0
H-139	OI 57	Z2085/putative exonuclease VIII, encoded within CP-933O	Cytoplasm	AE005346	4	0
H-158	OI 93	Z3327/unknown, encoded within CP-933V	Cytoplasm	AE005441	4	0
H-191	OI 71[d]	Z6060/putative Q anti-terminator encoded within CP-933P	Cytoplasm	AE006460	3	0
H-196	OI 93[d]	Z3334/unknown, encoded within CP-933V	Cytoplasm	AE005442	4	1
H-247	OI 57[d]	Z2100/unknown, encoded within CP-933O	Inner membrane	AE005347	4	0
H-255, -257	OI 36	Z0975/putative tail component of CP-933K	Inner membrane	AE005257	4	0
H-272	OI 79[d]	Z3097/putative peptidase—putative head-tail connector protein encoded within CP-933U	Inner membrane, cytoplasm	AE005420	4	0
Unknown						
H-2	OI 172	Z5897/unknown	Inner membrane	AE005660	4	0
H-21	OI 7	Z0251/unknown (uncharacterized protein conserved in bacteria)	Cytoplasm	AE005198	4	0
H-107	OI 142	Z5002/unknown (uncharacterized protein conserved in bacteria)	Cytoplasm	AE005584	4	0
H-119	OI 89	Z3271/unknown	Inner membrane	AE005436	4	0
H-123	OI 89	Z3269/unknown	Cytoplasm	AE005436	4	0
H-146	OI 140	Z4912/unknown	Cytoplasm	AE005576	3	0
H-162	OI 102	Z3616/unknown	Cytoplasm	AE005466	4	0
H-269	OI 48[d]	Z1606/unknown	Inner membrane	AE005309	4	0

[a] Putative functions of hypothetical proteins determined from the COGs database when available.
[b] Predicted by the PSORT/PSORT-B program.
[c] Sera from four patients with HUS and one healthy person were tested individually.
[d] Homologous proteins also found on other OIs.

of a gene encoding RhsH, a similar protein, were also detected during APEC infection of chickens by SCOTS (7).

Many nonphage ivi proteins were expressed from seven of the nine large OIs (>15 kb) that reportedly encode putative virulence factors (Table 4) (44). Besides intimin-γ from OI 148, IVIAT also identified a putative acyl coenzyme A (CoA) synthetase (fatty acid:CoA ligase), expressed from OI 138, which catalyzes the formation of fatty acyl-CoA, a substrate for phospholipid biosynthesis and enzymes of β-oxidation, and is involved in diverse functions such as protein transport, protein acylation, enzyme activation, cell signaling, and control of transcription (40); a putative inner membrane ABC-type transport permease expressed from OI 47 and functioning in cell wall biogenesis; a putative inner membrane ABC-type bacteriocin/

TABLE 4. ivi proteins expressed from OIs encoding putative virulence factors

OI	Putative virulence factor	ivi protein/function
OI 7	Macrophage toxin and a chaperone	Z0251/unknown
OI 28	An RTX-toxin-like exoprotein and transport system	Z0634/putative cytoplasmic membrane ABC-type bacteriocin/lantibiotic exporter
OI 43	Urease gene cluster	Z1214/a predicted esterase
OI 47	Adhesin and polyketide or fatty acid biosynthesis system	Z1554/putative ABC-type transporter
OI 48	Urease gene cluster	Z1606/unknown
OI 115	TTSS[a] and secreted proteins similar to *Samonella-Shigella* Inv-Spa host cell invasion proteins	None identified
OI 122	Two toxins and a PagC-like virulence factor	None identified
OI 138	Fatty acid biosynthesis system	Z4856/putative acyl-CoA synthetase/fatty acid ligase
OI 148	LEE proteins	Eae/γ-intimin/attaching and effacing

[a] TTSS, type III secretion system.

lantibiotic exporter expressed from OI 28 that functions in the export of large molecules such as proteins and peptides and is homologous to ATP-binding proteins of ABC transporters and toxin secretion systems of several pathogens, including *Pseudomonas putida*, *Salmonella enterica* serovar Typhi, and *V. cholerae*; a cytoplasmic esterase of the α-β hydrolase superfamily expressed from OI 43; a conserved cytoplasmic protein of unknown function expressed from OI 7; and an inner membrane protein of unknown function expressed from OI 48. Interestingly, IVIAT did not identify clones expressing putative virulence factors encoded on OI 115 and OI 122 (Table 4). Perhaps these proteins are expressed equally in vitro and in vivo, resulting in the removal of corresponding reactive antibodies during adsorption, or these proteins are not immunogenic, or antibodies to these proteins are short-lived.

ivi proteins expressed from pO157. ivi proteins expressed from pO157 (Table 3) included the previously discussed TagA and MsbB2; SopA, an ATPase that accurately partitions low-copy-number F plasmids into daughter cells (also identified during APEC infection of chickens) (7); a putative nickase associated with plasmid maintenance; and a putative hemolysin expression-modulating protein, a homolog (90% amino acid identity) of the *E. coli* regulator Hha (3), which complexes with the nucleoid-associated universal regulator protein H-NS and governs expression of the *hly* operon in response to changes in temperature and osmolarity (32). In addition, Hha has also been shown to repress the LEE-encoded regulator (Ler) in *E. coli* O157, thereby causing reduced expression of the *esp* operon encoding the LEE translocator proteins EspA, EspB, and EspD (49).

A majority of clones expressing ivi proteins reacted specifically and broadly with HUS convalescent-phase serum samples from individual patients. ivi proteins for practical applications, such as the development of diagnostic markers, vaccines, and drugs, should ideally be expressed strongly during infection and evoke robust immune responses broadly in patients with *E. coli* O157 disease. The majority of the 223 positive clones identified earlier using pooled adsorbed HUS convalescent-phase sera reacted with each of the four individual serum samples that made up the pool but not with a control serum sample taken from a healthy pediatric patient (Tables 1, 2, and 3). However, 15 ivi proteins expressed from the backbone and four ivi proteins expressed from OIs reacted differentially with individual patient serum samples. We are cur-

TABLE 5. O157 backbone ivi proteins expressed in vitro as identified by proteomic analysis

Protein no.	O157 ivi protein	Result with 6 M urea and 1% SDS denaturing solution[a]		% Protein abundance
		Run 1	Run 2	
1	Z4279 transketolase 1 isozyme (*tktA*)	+	+	0.49
2	Z5747 GroES, 10-kDa chaperone binds to Hsp60 (*mopB*)	+	+	0.32
3	Z4263 fructose-bisphosphate aldolase, class II (*fba*)	+	+	0.28
4	Z4540 degrades sigma 32, integral membrane peptidase, cell division (*hflB*)	+	−	0.17
5	Z4616 acetyl-CoA carboxylase, biotin carboxylase subunit (*accC*)	+	−	0.14
6	Z4228 lysine tRNA synthetase, constitutive suppressor of ColE1 (*lysS*)	+	+	0.13
7	Z0176 2,3,4,5-tetrahydropyridine-2-carboxylate *N*-succinyltransferase (*dapD*)	+	+	0.11
8	Z3999 alanyl-tRNA synthetase (*alaS*)	+	+	0.11
9	Z2789 putative thiosulfate sulfur transferase	+	−	0.10
10	Z3827 serine hydroxymethyltransferase (*glyA*)	+	+	0.09
11	Z5977 hyperosmotically inducible periplasmic protein (*osmY*)	+	+	0.08
12	Z3777 histidine tRNA synthetase (*hisS*)	+	+	0.07
13	Z5955 methyl-accepting chemotaxis protein I, serine sensor receptor (*tsr*)	+	+	0.06
14	Z3491 ribonucleoside diphosphate reductase 1, beta subunit, B2 (*nrdB*)	+	+	0.06
15	Z4043 methyl-directed mismatch repair (*mutS*)	−	+	0.05
16	Z4478 ORF, hypothetical protein (*yhaF*)	+	−	0.05
17	Z0998 DNA repair excision nuclease subunit B (*uvrB*)	−	+	0.05
18	Z5392 protein disulfide isomerase I, essential for cytochrome (*dsbA*)	+	+	0.04

[a] Two 4-h runs were done using this denaturing condition. Each yielded more than 300 proteins.

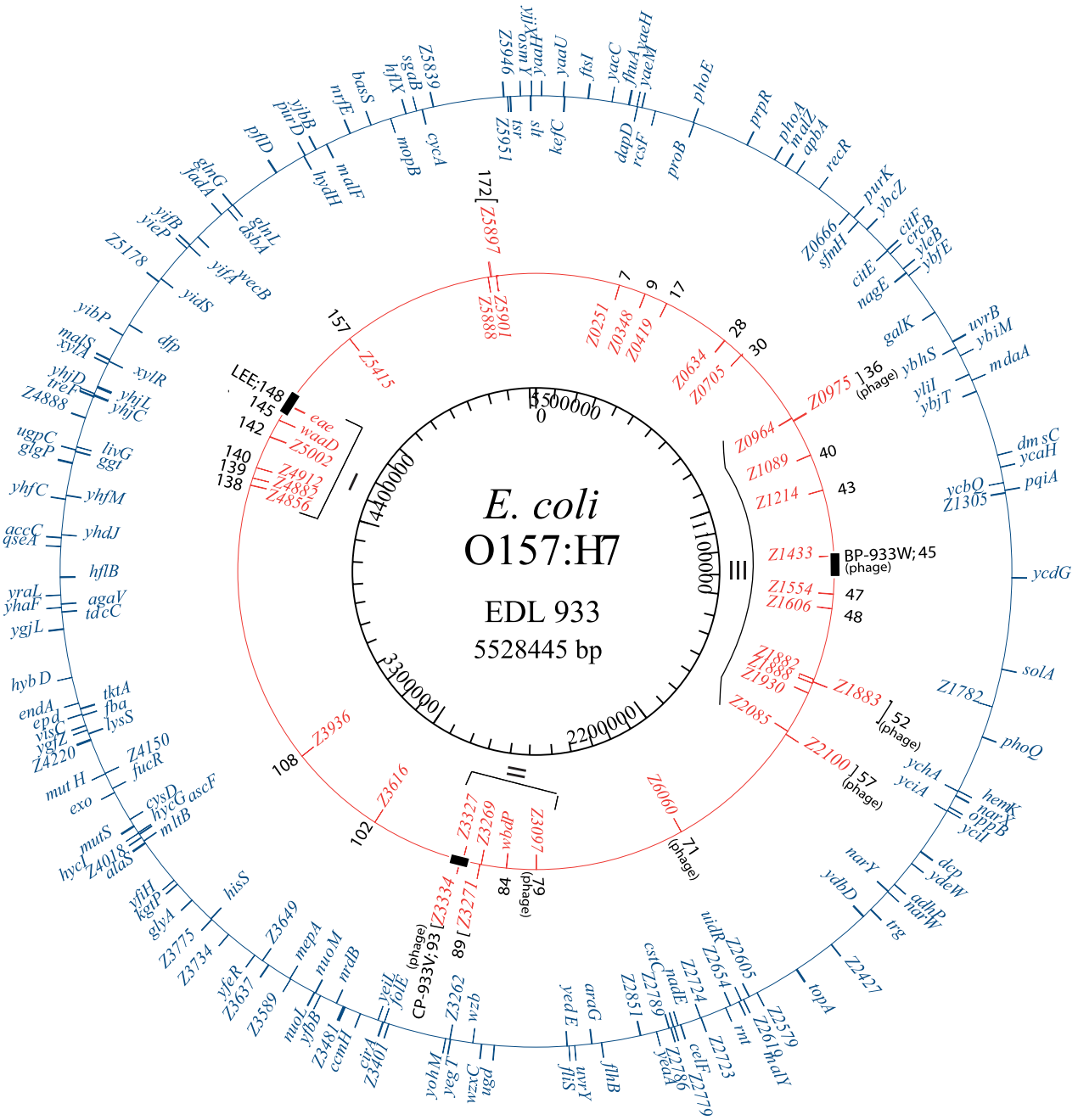

FIG. 3. Graphical representation of the locations of ivi genes on the chromosome of *E. coli* O157 strain EDL933. Outer and inner circles show the positions of ivi genes on the backbone and OIs, respectively. The black numbers on the outside of the inner circle refer to individual OIs (44). Individual OI genes, phage-associated genes, the LEE (encoding intimin-γ), CP-933V (encoding Stx1), and BP-933W (encoding Stx2) are also shown. OI groups, I, II, and III are indicated (see the text). The innermost circle shows the scale in base pairs. This figure was created using Genvision software from DNASTAR.

rently investigating via PCR whether the failure of individual patients to respond to a particular ivi protein is attributable to heterogeneity of cognate isolates. Also, 22 backbone (Table 2) and 2 *E. coli* O157-specific (Table 3) ivi proteins reacted with the control serum. We speculate that this may be due to cross-reacting antibodies in the control sera or to the presence of preexisting antibodies to O157 proteins from prior, unrecognized infection. Studies are ongoing to compare the reactivities of sera from healthy individuals of different age groups to the ivi antigens.

ivi proteins expressed from OIs and pO157 were not among the 300 O157 proteins most highly expressed during in vitro growth. The central premise of IVIAT is that the proteins identified are expressed specifically during infection but not

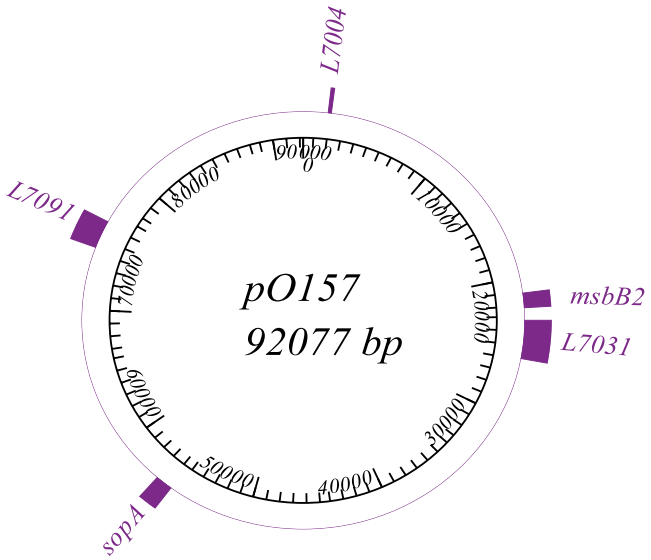

FIG. 4. Graphical representation of the locations of ivi genes on the pO157 plasmid of *E. coli* O157 strain EDL933. The outer circle shows the positions of ivi genes (magenta), and the inner circle, the scale in base pairs. This figure was created using Genvision software from DNASTAR.

during growth under standard laboratory conditions. Proteomic analysis using ESIμLC-MS/MS confirmed that none of the 37 ivi proteins expressed from OIs and none of the 5 expressed from pO157 were among the 300 O157 proteins most highly expressed during growth in LB broth (data not shown). This was not entirely expected, because some of the *E. coli* O157-specific proteins are reportedly expressed, at least to some degree, in vitro (36). We speculate that, owing to low-level expression of such proteins during in vitro culture, an MS run of longer duration would be required for their identification.

In contrast, 18 of 181 backbone ivi proteins were expressed sufficiently in LB broth for detection by proteomic analysis (Table 5). Of these 18 backbone ivi proteins expressed in vitro, 12 were identified by ESIμLC-MS/MS during the course of both runs; 6 of which were weakly expressed at a percent protein abundance ranging from 0.09 to 0.04%, and the remaining 6 were expressed in only one run (Table 5). We hypothesize that these 18 proteins are expressed at higher levels during human infection than during growth in LB broth and attribute their identification by IVIAT to the fact that low-level protein expression during in vitro growth may not effectively deplete HUS convalescent-phase sera of antibodies against these ivi proteins during absorption.

Graphical representations of the locations of ivi genes on the *E. coli* O157 chromosome and on the pO157 plasmid are shown in Fig. 3 and 4, respectively. ivi genes included 181 of 4,029 (4.5%) open reading frames (ORFs) in the backbone, 37 of 1,387 (2.7%) ORFs in the OIs, and 5 of 100 (5%) ORFs in pO157 sequences of the EDL933 genome (44).

The 181 backbone-specific ivi genes were distributed uniformly on the *E. coli* O157 chromosome; however, several of the 37 OI-specific ivi genes appeared to localize in three discrete OI groups (a group contained four or more OIs, with each separated by five or fewer intervening OIs). Although in most cases only one ivi gene mapped to an individual OI, there were instances where multiple ivi genes (two or more) mapped within the same OI (OI 36, OI 52, OI 57, OI 89, OI 93). The five ivi genes that mapped to pO157 are also shown in Fig. 4.

The apparent grouping of OIs expressing ivi proteins raises the possibility that ivi proteins (and other proteins) expressed from OIs within a particular group might act in concert to optimally influence a specific function. Particularly interesting was the fact that group I, which included OI 148, expressing the adhesin intimin-γ, also included OI 145, expressing the glycosyltransferase WaaD, one of the many enzymes involved in the biosynthesis of LPS core oligosaccharide. The facts that WaaD is expressed from the same operon as WaaI (another enzyme that functions in LPS core oligosaccharide biosynthesis) and that *E. coli* O157 *waaI* deletion mutants are hyperadherent to cultured intestinal epithelial cells (54) may suggest that during human infection, intimin-γ and *E. coli* O157 LPS (and possibly other ivi and non-ivi proteins, expressed from OIs within this group) might act in concert to modulate the adherence of *E. coli* O157 to human epithelial cells. It will be interesting to test this hypothesis experimentally and also to determine whether proteins in OI groups II and III might be functionally related as well.

In conclusion, IVIAT identified 223 O157 proteins expressed in vivo during human infection, several of which were unique to this study. Judged by our results, IVIAT enables identification both of proteins expressed specifically during human infection but not during growth under standard laboratory conditions and of proteins expressed at significantly higher levels in vivo than in vitro. Although IVIAT for *E. coli* O157 was validated by the identification of previously identified potential *E. coli* O157 virulence factors, prior infection with *E. coli* O157 does not necessarily produce full protection from subsequent reinfection (1). This may reflect suboptimal antibody responses to protective antigens, and we hypothesize that robust expression and optimal delivery of relevant ivi proteins (and other O157 antigens) to the mucosal immune system might engender more-protective immune responses. Preliminary experiments demonstrated that all of the 223 reactive clones also reacted with pooled adsorbed sera from patients who had recovered from hemorrhagic colitis (data not shown), suggesting that similar pathogenic mechanisms may be operating in this illness and HUS.

IVIAT provides a "snapshot" of O157 protein expression during infection and a glimpse of the possible mechanisms by which this pathogen might counter host defenses and adapt and establish itself within the human gut to cause disease. Studies directed toward the characterization of the role of ivi proteins in *E. coli* O157 pathogenesis are currently under way. The identification of ivi genes unique to diverse *E. coli* O157 isolates, as well as identification of those unique to non-O157 EHEC or to Shiga-toxigenic *E. coli* (STEC), which lacks LEE but is pathogenic to humans, and of ivi genes shared between EHEC and EPEC (unpublished data), augurs well for the future development of diagnostic tests for EHEC and STEC infection, as well as for the development of common drugs and vaccines against EHEC, STEC, and EPEC.

ACKNOWLEDGMENTS

This work was supported by grants R03 AI53700-01 and R21 AI055963 (to I.T.K.), R01 DE13523 (to M.H.), and R01 DK52081 and R01 AI47499 (to P.I.T.) from the National Institutes of Health.

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Editor: A. D. O'Brien