Microarray Analysis of the Ler Regulon in Enteropathogenic and Enterohaemorrhagic *Escherichia coli* Strains

Lewis E. H. Bingle, Chrystala Constantinidou, Robert K. Shaw, Md. Shahidul Islam, Mala Patel, Lori A. S. Snyder, David J. Lee, Charles W. Penn, Stephen J. W. Busby, Mark J. Pallen

School of Biosciences, University of Birmingham, Edgbaston, Birmingham, United Kingdom

**Abstract**

The type III protein secretion system is an important pathogenicity factor of enteropathogenic and enterohaemorrhagic *Escherichia coli* pathotypes. The genes encoding this apparatus are located on a pathogenicity island (the locus of enterocyte effacement) and are transcriptionally activated by the master regulator Ler. In each pathotype Ler is also known to regulate genes located elsewhere on the chromosome, but the full extent of the Ler regulon is unclear, especially for enteropathogenic *E. coli*. The Ler regulon was defined for two strains of *E. coli*: E2348/69 (enteropathogenic) and EDL933 (enterohaemorrhagic) in mid and late log phases of growth by DNA microarray analysis of the transcriptomes of wild-type and ler mutant versions of each strain. In both strains the Ler regulon is focused on the locus of enterocyte effacement — all major transcriptional units of which are activated by Ler, with the sole exception of the *LEE1* operon during mid-log phase growth in E2348/69. However, the Ler regulon does extend more widely and also includes unlinked pathogenicity genes: in E2348/69 more than 50 genes outside of this locus were regulated, including a number of known or potential pathogenicity determinants; in EDL933 only 4 extra-LEE genes, again including known pathogenicity factors, were activated. In E2348/69, where the Ler regulon is clearly growth phase dependent, a number of genes including the plasmid-encoded regulator operon *perABC*, were found to be negatively regulated by Ler. Negative regulation by Ler of *PerC*, itself a positive regulator of the *ler* promoter, suggests a negative feedback loop involving these proteins.

Citation: Bingle LEH, Constantinidou C, Shaw RK, Islam MS, Patel M, et al. (2014) Microarray Analysis of the Ler Regulon in Enteropathogenic and Enterohaemorrhagic *Escherichia coli* Strains. PLoS ONE 9(1): e80160. doi:10.1371/journal.pone.0080160

**Editor:** David A. Rasko, University of Maryland School of Medicine, United States of America

**Received:** August 24, 2012; **Accepted:** October 9, 2013; **Published:** January 14, 2014

**Copyright:** © 2014 Bingle et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** This work was funded by a grant from the BBSRC (www.bbsrc.ac.uk). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

* E-mail: m.pallen@warwick.ac.uk

† Current address: Faculty of Applied Sciences, University of Sunderland, Sunderland, United Kingdom

‡ Current address: Division of Microbiology & Infection, University of Warwick, Coventry, United Kingdom

§ Current address: Department of Biotechnology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

¶ Current address: School of Life Sciences, Kingston University, Kingston upon Thames, United Kingdom

* These authors contributed equally to this work.

**Introduction**

Enteropathogenic (EPEC) and enterohaemorrhagic (EHEC) *Escherichia coli* are two pathotypes of this important gastrointestinal bacterium that can cause serious diarrheal disease in humans [1]. Many EHEC and EPEC strains possess a type III secretion system (T3SS) encoded by a pathogenicity island called the locus of enterocyte effacement (LEE) that is also found in the related bacterium *Citrobacter rodentium*, a mouse pathogen that is widely used as a model for the EHEC and EPEC strains [2]. Pathogenicity factors encoded within the LEE, specifically the type III secretion system and secreted effector proteins, are responsible for formation of the attaching and effacing (AE) lesion on the gut epithelium that is characteristic of these strains and required for intimate attachment of the bacteria [3]. The 41 genes of the LEE are arranged in 5 major polycistronic operons called *LEE1*-5 along with a number of smaller transcriptional units [4]. Attaching and effacing pathogens, including EPEC strains such as E2348/69, O157:H7 EHEC strains and non-0157 EHEC strains, have distinct evolutionary histories but carry an overlapping core repertoire of pathogenicity genes, including the LEE and many effector genes outside the LEE, that have been acquired via horizontal gene transfer [5,6,7]. However, there are significant differences in overall pathogenicity between EHEC and EPEC strains, for example EHEC strains cause a more severe bloody diarrheal disease (haemorrhagic colitis) that is often accompanied by the life threatening complication, haemolytic uraemic syndrome (HUS) [8]. Such differences are presumably mainly determined by the differing contributions of the extra-LEE factors. Examples include differing arrays of T3SS effector proteins and the fact that the EHEC genome encodes a Shiga-like toxin responsible for serious pathology in the human host, while EPEC does not [8].

In addition to variation in the genomic arsenal of determinants, appropriate control of gene transcription may be critical in optimising pathogenicity [9,10]. Type III secretion systems (T3SS) are generally acquired through horizontal gene transfer and
Microarray Analysis of the Ler Regulon

We constructed two validated mutant strains of *E. coli*: LBEC1 (EDL933 Δler) and LBEC2 (E2348/69 Δler), grew cultures of WT and parental mutant strains under conditions known to be inducing for the LEE to two different growth phases (mid and late log phase), harvested RNA and used this to perform microarray analysis of the transcriptomes. Microarray data has been deposited with the GEO database (http://www.ncbi.nlm.nih.gov/geo) with accession code GSE38076.

**Enteropathogenic *E. coli***

In mid-log phase cells a total of 85 genes are transcriptionally regulated: 62 genes, at 14 different loci, are activated by Ler (table 1) while 23 genes, at 6 loci, are repressed by Ler (table 2). Of the activated genes 49 (79%) are carried on or directly adjacent to mobile genetic elements (MGEs: prophage, integrative element or plasmid), while 11 of the repressed genes (48%) are carried on MGEs. If one compares the genes that are activated and repressed, two repressed genes (E2348C_0004 and E2348C_2114) are potentially expressed from promoters that are immediately divergent from an activated promoter.

In late log phase cells 97 genes in total are regulated by Ler. Of these, 65 genes at 23 genetic locations are activated, of which 62 genes (73%) are carried on or directly adjacent to MGEs (table 5). Twelve genes are repressed by Ler, of which only 1 is adjacent to a MGE (table 4).

The strongest activation was generally observed for LEE genes, with the mostly high activated genes being *eae* at mid-log phase (58-fold) and *espC* in late log phase (100-fold). Extra LEE genes with comparable levels of activation included *espC* (mid-log only), *pagP* and the gene encoding the T3SS secreted effector NleA. The maximum fold repression observed outside of the LEE was approximately 9-fold in mid-log phase (*fimD*) and approximately 6-fold in late log phase cells (*chuT-hmaV* heme utilization operon).

**Enterohaemorrhagic *E. coli***

In mid-log phase cells, only one gene passed the Benjamini and Hochberg MTC filter as being repressed (2-fold) by Ler. This was *Z2974* on prophage CP-933T, encoding an unknown protein.

In late-log phase cells, 39 genes were found to be transcriptionally activated by Ler (2-fold or more; table 5). Thirty-five of these genes are within the LEE (representing all major transcriptional units; activation between 4 and 32-fold). The remaining 4 extra-LEE activated genes encode: *StcE* (4-fold), *EtpC* (3-fold), *SfaP* (5-fold) and the putative cytochrome *YhaI* (36-fold). The *stcE* and *espC* genes are located on plasmid pO157; *SfaP* is prophage-encoded and yhaI is not associated with a mobile genetic element.

**Discussion**

It is clear that in both the EPEC and EHEC strains of *E. coli* examined here, the LEE is the primary target for Ler activation: all major transcriptional units of the LEE are regulated by Ler, although the regulation of LEE1 is growth phase dependent in EPEC, as noted below. Otherwise, in EPEC the Ler regulon is quite small, covering about 2% of the genome; in EHEC the regulon is even smaller and contains very few genes outside of the LEE. As the positive regulatory activity of Ler is known to be due to antagonism of H-NS repression (where studied) we would predict that all activated members of the Ler regulon are repressed by H-NS. However the H-NS regulon is very large and clearly not all H-NS repressed genes are activated by Ler [38]. An important question that therefore remains to be answered is: what provides specificity to Ler regulation? The specificity of action that we have observed (i.e. most of the strongly regulated genes are located within the LEE) is in agreement with the observations of Abe *et al.* relating to EHEC [36]. This specificity is consistent with Ler binding to a specific DNA structural motif, via an indirect readout...
| Systematic Gene Name | Fold Activation | Common Gene Name | Gene Product | MGE |
|----------------------|----------------|------------------|--------------|-----|
| E2348C_0081          | 6              | leuO             | DNA-binding transcriptional activator |     |
| E2348C_0153          | 4              | -                | predicted fimbrial protein |     |
| E2348C_0523          | 9              | pagP             | palmitoyl transferase for Lipid A | PP2 |
| E2348C_0683          | 4              | -                | hypothetical protein | PP2 |
| E2348C_0684          | 21             | SfpA             | systemic factor protein A-like protein | PP2 |
| E2348C_1040          | 2              | -                | T3SS secreted effector NleI/NleG homolog | PP4 |
| E2348C_1442          | 13             | -                | T3SS secreted effector NleA/Espl homolog | PP6 |
| E2348C_1444          | 2              | -                | T3SS secreted effector NleH homolog | PP6 |
| E2348C_2076          | 4              | yedR             | hypothetical protein | IE3 |
| E2348C_2111          | 3              | -                | hypothetical protein | IE3 |
| E2348C_2112          | 2              | -                | predicted glycosyl transferase | IE5 |
| E2348C_2705          | 3              | -                | extracellular serine protease EspC | IE5 |
| E2348C_2916          | 3              | -                | T3SS secreted effector EspG homolog | IE5 |
| E2348C_2917          | 3              | -                | hypothetical protein | IE5 |
| E2348C_2918          | 3              | -                | hypothetical protein | IE5 |
| E2348C_2920          | 27             | -                | hypothetical protein | IE5 |
| E2348C_3262          | 2              | -                | predicted acyl-CoA synthase |     |
| E2348C_3839          | 5              | yiaR             | predicted Fe-containing alcohol dehydrogenase |     |
| E2348C_3929          | 2              | yicJ             | predicted transporter | ±LEE |
| E2348C_3930          | 9              | espF             | LEE-encoded effector EspF | LEE |
| E2348C_3931          | 52             | orf29            | component of T3SS, SsaH family | LEE |
| E2348C_3932          | 42             | escF             | T3SS structure protein EscF | LEE |
| E2348C_3933          | 45             | cesD2            | chaperone CesD2 | LEE |
| E2348C_3934          | 18             | espB             | translocator EspB | LEE |
| E2348C_3935          | 39             | espD             | translocator EspD | LEE |
| E2348C_3936          | 42             | espA             | translocator EspA | LEE |
| E2348C_3937          | 36             | sepL             | secretion switching protein SepL | LEE |
| E2348C_3938          | 32             | escD             | T3SS structure protein EscD | LEE |
| E2348C_3939          | 58             | eae              | intimin Eae | LEE |
| E2348C_3940          | 26             | cesT             | chaperone CesT | LEE |
| E2348C_3941          | 34             | tir              | translocated intimin receptor Tir | LEE |
| E2348C_3942          | 44             | map              | LEE-encoded effector Map | LEE |
| E2348C_3943          | 22             | cesF             | chaperone CesF | LEE |
| E2348C_3944          | 21             | espH             | LEE-encoded effector EspH | LEE |
| E2348C_3945          | 26             | sepQ             | T3SS structure protein SepQ | LEE |
| E2348C_3946          | 21             | orf16            | hypothetical protein | LEE |
| E2348C_3947          | 16             | orf15            | hypothetical protein | LEE |
| E2348C_3948          | 11             | escN             | translocator EscN | LEE |
| E2348C_3949          | 17             | escV             | translocator EscV | LEE |
| E2348C_3950          | 12             | mpc              | regulator Mpc | LEE |
| E2348C_3951          | 13             | espZ             | LEE-encoded effector EspZ | LEE |
| E2348C_3952          | 25             | rofB             | chaperone of T3SS RofB | LEE |
| E2348C_3953          | 24             | escJ             | T3SS structure protein EscJ | LEE |
| E2348C_3954          | 28             | sepD             | secretion switching protein SepD | LEE |
| E2348C_3955          | 29             | escC             | T3SS structure protein EscC | LEE |
| E2348C_3956          | 23             | cesD             | chaperone CesD | LEE |
| E2348C_3957          | 14             | grlA             | positive regulator GrlA | LEE |
| E2348C_3958          | 7              | grlR             | negative regulator GrlR | LEE |
### Table 1. Cont.

| Systematic Gene Name | Fold Activation | Common Gene Name | Gene Product | MGE |
|----------------------|----------------|------------------|--------------|-----|
| E2348C_3959          | 9              | rorf3            | hypothetical protein | LEE |
| E2348C_3960          | 3              | iraP             | T3SS structure protein EscU | LEE |
| E2348C_3968          | 21             | ler              | transcription regulator Ler | LEE |
| E2348C_3970          | 12             | espG             | LEE-encoded effector EspG | LEE |
| E2348C_3971          | 50             | rorf1            | hypothetical protein | LEE |
| E2348C_4274          | 2              | -                | predicted transporter |       |
| E2348C_4348          | 2              | malF             | maltose transporter subunit |       |
| E2348C_4349          | 2              | malE             | maltose transporter subunit |       |
| E2348C_4350          | 3              | malK             | maltose transporter subunit |       |
| E2348C_4351          | 3              | lamB             | maltose outer membrane porin (maltoporin) |       |
| E2348C_4442          | 3              | eptA             | predicted metal dependent hydrolase EptA |       |
| pMAR2_006            | 3              | -                | putative glutamate:gamma-aminobutyrate antiporter | pMAR2 |
| pMAR2_007            | 2              | -                | putative glutamate racemase | pMAR2 |

Fold activation shows expression in ler / ler" cells. MGE, mobile genetic element; IE, integrative element; LEE, locus of enteroceptor effacement; ± LEE, directly adjacent to the LEE; pMAR2, plasmid. For each gene reported, \( t \)-test P-value was less than 0.05.

doi:10.1371/journal.pone.0080160.t001

### Table 2. EPEC genes repressed 2-fold or more by Ler at mid-log phase (OD\(_{600}\) = 0.4).

| Systematic Gene Name | Fold Repression | Common Gene Name | Gene Product | MGE |
|----------------------|----------------|------------------|--------------|-----|
| E2348C_0084          | 2              | -                | fruR leader peptide |       |
| E2348C_0318          | 2              | iraP             | hypothetical protein | IE2 |
| E2348C_1098          | 2              | -                | hypothetical protein | IE2 |
| E2348C_1922          | 2              | yeaQ             | conserved inner membrane protein |       |
| E2348C_2034          | 3              | sdiA             | DNA-binding transcriptional activator |       |
| E2348C_2114          | 2              | -                | hypothetical protein | IE3 |
| E2348C_3959          | 9              | rorf3            | hypothetical protein | LEE |
| E2348C_3960          | 3              | iraP             | T3SS structure protein EscU | LEE |
| E2348C_3970          | 12             | espG             | LEE-encoded effector EspG | LEE |
| E2348C_3971          | 50             | rorf1            | hypothetical protein | LEE |
| E2348C_4274          | 2              | -                | predicted transporter |       |
| E2348C_4348          | 2              | malF             | maltose transporter subunit |       |
| E2348C_4349          | 2              | malE             | maltose transporter subunit |       |
| E2348C_4350          | 3              | malK             | maltose transporter subunit |       |
| E2348C_4351          | 3              | lamB             | maltose outer membrane porin (maltoporin) |       |
| E2348C_4442          | 3              | eptA             | predicted metal dependent hydrolase EptA |       |
| pMAR2_006            | 3              | -                | putative glutamate:gamma-aminobutyrate antiporter | pMAR2 |
| pMAR2_007            | 2              | -                | putative glutamate racemase | pMAR2 |

Fold repression shows expression in ler / ler" cells. MGE, mobile genetic element; IE, integrative element; pMAR2, plasmid. For each gene reported, \( t \)-test P-value was less than 0.05.

doi:10.1371/journal.pone.0080160.t002
Table 3. EPEC genes activated 2-fold or more by Ler at late-log phase (OD$_{600} = 0.9$).

| Systematic Gene Name | Fold Activation | Common Gene Name | Gene Product | MGE |
|----------------------|-----------------|------------------|--------------|-----|
| E234GC_0081          | 6               | _leuO_           | DNA-binding transcriptional activator |     |
| E234GC_0153          | 5               | -                | predicted fimbrial protein |     |
| E234GC_0523          | 15              | _pagP_           | palmitoyl transferase for Lipid A | PP2 |
| E234GC_0683          | 3               | -                | hypothetical protein | PP2 |
| E234GC_0684          | 35              | -                | SfpA (systemic factor protein A)-like protein | PP2 |
| E234GC_0685          | 2               | -                | predicted late gene regulator | PP2 |
| E234GC_0718          | 2               | -                | T3SS secreted effector NleH homolog | PP2 |
| E234GC_0723          | 3               | -                | T3SS secreted effector EspJ homolog | PP2 |
| E234GC_1040          | 2               | -                | T3SS effector-like protein NleB homolog | PP2 |
| E234GC_1041          | 2               | -                | T3SS secreted effector NleA/EspI homolog | PP6 |
| E234GC_1442          | 15              | -                | T3SS secreted effector NleE homolog | PP6 |
| E234GC_1444          | 2               | -                | T3SS secreted effector NleH homolog | PP6 |
| E234GC_1445          | 2               | -                | T3SS secreted effector NleF homolog | PP6 |
| E234GC_1481          | 2               | -                | yciW         | predicted oxidoreductase |
| E234GC_2065          | 2               | _rcsA_           | DNA-binding transcriptional activator, co-regulator with RcsB |     |
| E234GC_2076          | 6               | _yedR_           | hypothetical protein |     |
| E234GC_2105          | 2               | -                | hypothetical protein | IE3 |
| E234GC_2111          | 5               | -                | hypothetical protein | IE3 |
| E234GC_2112          | 4               | -                | hypothetical protein | IE3 |
| E234GC_2113          | 2               | -                | hypothetical protein | IE3 |
| E234GC_2129          | 3               | _pduF_           | propanediol diffusion facilitator |     |
| E234GC_2396          | 3               | _ais_            | hypothetical protein |     |
| E234GC_2607          | 3               | _cysA_           | sulfate/thiosulfate transporter subunit |     |
| E234GC_2608          | 3               | _cysW_           | sulfate transport system permease W protein; membrane component of ABC superfamily |     |
| E234GC_2609          | 3               | _cysU_           | sulfate, thiosulfate transport system permease T protein; membrane component of ABC superfamily |     |
| E234GC_2610          | 2               | _cysP_           | thiosulfate transporter subunit |     |
| E234GC_2705          | 5               | -                | predicted glycosyl transferase |     |
| E234GC_2915          | 39              | _espC_           | extracellular serine protease EspC | IE5 |
| E234GC_2916          | 4               | -                | T3SS secreted effector EspG homolog | IE5 |
| E234GC_2917          | 5               | -                | hypothetical protein | IE5 |
| E234GC_2918          | 3               | -                | hypothetical protein | IE5 |
| E234GC_2920          | 25              | -                | hypothetical protein | IE5 |
| E234GC_3020          | 3               | _cysC_           | adenosine 5’-phosphosulfate kinase |     |
| E234GC_3021          | 2               | _cysN_           | sulfate adenylyltransferase, subunit 1 |     |
| E234GC_3022          | 3               | _cysD_           | sulfate adenylyltransferase, subunit 2 |     |
| E234GC_3025          | 2               | _cysV_           | 3’-phosphoadenosine 5’-phosphosulfate reductase |     |
| E234GC_3026          | 3               | _cysI_           | sulfite reductase, β subunit; NAD(P)-binding, heme-binding |     |
| E234GC_3027          | 3               | _cysJ_           | sulfite reductase, alpha subunit, flavoprotein |     |
| E234GC_3262          | 2               | -                | predicted acyl-CoA synthase |     |
| E234GC_3264          | 2               | -                | predicted acyl carrier protein |     |
| E234GC_3839          | 9               | _yjaY_           | predicted Fe-containing alcohol dehydrogenase |     |
| E234GC_3929          | 4               | _yicJ_           | predicted transporter | LEE |
| E234GC_3930          | 7               | _espF_           | LEE-encoded effector EspF | LEE |
| E234GC_3931          | 100             | _orf29_          | component of T3SS, SsaH family | LEE |
| E234GC_3932          | 96              | _escF_           | T3SS structure protein EscF | LEE |
| E234GC_3933          | 73              | _cesD2_          | chaperone CesD2 | LEE |
| E234GC_3934          | 20              | _espB_           | translocator EspB | LEE |
mechanism, as suggested by an NMR analysis of Ler C-terminal domain-DNA complexes [39]. While some studies have shown evidence for specific binding of LEE promoters by Ler, the Chip-CHIP analysis of Abe et al. suggested that Ler was binding extensively (although not evenly) across the Sakai genome and the authors concluded that Ler has a low binding specificity [36].

Many but not all of the extra LEE members of the EPEC Ler regulon are located on mobile genetic elements (MGEs) and it is particularly striking that Ler negatively regulates a disproportionately high number of plasmid-borne genes, at least in mid-log phase EPEC: 9 genes from 3 different operons (10% of the total of 90 genes) on plasmid pMAR2 are shown to be regulated, while only 0.3% of the chromosomal genes (14 genes) are repressed. However by late log phase, no plasmid-borne genes are repressed by Ler. Similarly it is striking that 4 of the 5 extra-LEE genes found to be Ler-regulated in EHEC (likely members of the same operon) are located on a MGE (plasmid or prophage). In both bacteria the GC contents of chromosome is much higher in the region directly adjacent to the LEE; pMAR2, plasmid. For each gene reported, t-test P-value was less than 0.05.

doi:10.1371/journal.pone.0080160.t003

| Systematic Gene Name | Fold Activation | Common Gene Name | Gene Product | MGE |
|----------------------|----------------|------------------|--------------|-----|
| E2348C_3935          | 66             | espD             | translocator EspD | LEE |
| E2348C_3936          | 97             | espA             | translocator EspA | LEE |
| E2348C_3937          | 100            | sepL             | secretion switching protein SepL | LEE |
| E2348C_3938          | 80             | escD             | T3SS structure protein EscD | LEE |
| E2348C_3939          | 100            | eoe              | intimin Eae | LEE |
| E2348C_3940          | 62             | cesT             | chaperone CesT | LEE |
| E2348C_3941          | 39             | tir              | translated intimin receptor Tir | LEE |
| E2348C_3942          | 73             | map              | LEE-encoded effector Map | LEE |
| E2348C_3943          | 79             | cesF             | chaperone CesF | LEE |
| E2348C_3944          | 38             | espH             | LEE-encoded effector EspH | LEE |
| E2348C_3945          | 54             | sepQ             | T3SS structure protein SepQ | LEE |
| E2348C_3946          | 37             | orf16            | hypothetical protein | LEE |
| E2348C_3947          | 25             | orf15            | hypothetical protein | LEE |
| E2348C_3948          | 14             | escN             | translocator EscN | LEE |
| E2348C_3949          | 39             | escV             | translocator EscV | LEE |
| E2348C_3950          | 27             | mpc              | regulator Mpc | LEE |
| E2348C_3951          | 15             | espZ             | LEE-encoded effector EspZ | LEE |
| E2348C_3952          | 39             | rofB             | chaperone of T3SS RofB | LEE |
| E2348C_3953          | 47             | escJ             | T3SS structure protein EscJ | LEE |
| E2348C_3954          | 46             | sepD             | secretion switching protein SepD | LEE |
| E2348C_3955          | 46             | escC             | T3SS structure protein EscC | LEE |
| E2348C_3956          | 45             | cesD             | chaperone CesD | LEE |
| E2348C_3957          | 47             | grlA             | positive regulator GrlA | LEE |
| E2348C_3958          | 28             | grlR             | negative regulator GrlR | LEE |
| E2348C_3959          | 44             | rofI             | hypothetical protein | LEE |
| E2348C_3960          | 13             | escU             | T3SS structure protein EscU | LEE |
| E2348C_3961          | 9              | escT             | T3SS structure protein EscT | LEE |
| E2348C_3962          | 9              | escS             | T3SS structure protein EscS | LEE |
| E2348C_3963          | 10             | escR             | T3SS structure protein EscR | LEE |
| E2348C_3964          | 9              | orfS             | component of T3SS | LEE |
| E2348C_3965          | 6              | orfA             | component of T3SS | LEE |
| E2348C_3966          | 7              | orf3             | component of T3SS | LEE |
| E2348C_3967          | 6              | orf2             | component of T3SS | LEE |
| E2348C_3968          | 26             | ler              | transcription regulator Ler | LEE |
| E2348C_3970          | 12             | espG             | LEE-encoded effector EspG | LEE |
| E2348C_3971          | 58             | rofI             | hypothetical protein | LEE |
| E2348C_4442          | 4              | eptA             | predicted metal dependent hydrolase EptA | LEE |
| pMAR2_097            | 3              | -                | putative glutamate racemase | pMAR2, plasmid |

Table 3. Cont.
slightly higher than that of the plasmid: EDL933 and E2348/69 chromosomes are 50.4 and 50.6% respectively, while pO157 and pMAR2 are 47.6% and 48%. As genes located on MGEs with lower GC content are selectively silenced by H-NS [40], in evolutionary terms Ler activation could have been a useful means to “liberate” the expression of newly acquired pathogenicity factors.

Across the genome, the Ler regulon is notably growth phase dependent in EPEC; in mid log phase (OD_{600} = 0.4) 27 extra-LEE genes are activated by Ler, while in late log phase (OD_{600} = 0.9) the number of activated extra-LEE genes is 43. In EPEC the regulation of the LEE1 operon, but not the other operons of the LEE, differs between mid-log and late-log growth phases: at late log phase, all 41 genes within the LEE are strongly activated by Ler, along with the flanking predicted sugar transporter gene yicJ, while at mid-log phase the 7 genes in the LEE1 operon before cofU are not strongly (>2-fold) regulated (Figure 1; note that we do not comment on the regulation of the ler gene itself as the coding sequence is partly deleted in the mutant). While it is possible that we have introduced some artefactual corruption of LEE1 regulation during mutation of ler, the observed activation of late-log phase cells suggests that there is no gross defect in the Ler regulatory circuit. This result indicates that the regulation of the LEE1 promoter is somewhat different to that of other LEE promoters, possibly due to a complex balance between Ler autoregulation and activation. It is noteworthy that, while previous reporter gene analysis of the LEE1 promoter has indicated that it is autorepressed by Ler, our results indicate that it may be activated, a difference that may reflect the growth phase dependence of the effects observed here [15]. No corresponding differential regulation of the LEE1 operon was observed in late log phase EHEC; in the mid-log phase cultures none of the LEE genes passed the MTC filter, but if the filter is not applied then LEE1 seems to be similarly regulated in mid-log and late-log phase cultures. While Sperandio et al. found that the LEE4 operon (espL-espF) was constitutively expressed at a high level in EHEC and insensitive to Ler regulation [20], we have found it to be clearly Ler-dependent in both EHEC and EPEC strains. The observed difference could have resulted from selection of a promoter fragment for reporter gene assays that lacks the full complement of H-NS binding sites.

There are a number of Ler-activated genes in the EPEC regulon that are outside of the LEE but may be involved in pathogenicity. As noted above, espG is already known to be ler-regulated and is one of the mostly highly (22-fold) activated genes in mid-log phase cells. PagP, the palmitoyl transferase for lipid A is strongly regulated at both mid and late log phases (9-fold and 15-fold respectively). Palmitoylated lipid A supposedly protects bacteria from host immune defences (e.g. CAMPs) and attenuates their activation through the TLR4 signal transduction pathway [41]. E2348C_0684, strongly regulated along with its downstream neighbour, encodes a SfpA (systemic factor protein A)-like protein: SfpA is a porin involved in systemic disease in Yersinia enterocolitica [42]. A homologue of sfpA (ECs0814) in the Sakai strain of EHEC was previously observed to be Ler regulated [36]. The rcsA gene, which encodes a positive regulator of the serotype-specific group I K (capsular) antigen is activated by Ler in late log phase, although not at the earlier growth point [43]. This may reflect an impact of capsule production on the intimate attachment of EPEC bacteria to the gut epithelium, however, no regulation of the wza promoter (target for RcsA in E. coli K-12) was apparent. It is worth noting at this point that a ler mutant of EPEC was previously found to be defective for colonisation of Caenorhabditis elegans [44]. This requirement for Ler was found to be independent of T3SS encoded by the LEE. This effect is presumably due to one or more of these extra-LEE members of the Ler regulon which are essential pathogenicity factors in a C. elegans infection but are not involved in T3S (and are not effectors delivered by the T3SS). Several non-LEE encoded effector genes, whose products are secreted via the T3SS, are Ler regulated in EPEC, including the operon of five genes from nleI/G to nleF [45]. Ler regulation of a homologue of nleA is already known to occur in EHEC [33] and a homolog of the espG gene located next to the espC gene which is also Ler regulated (see above). There is also clear evidence for the transcriptional regulation of nleH and espJ homologues at late log phase. While it may be unsurprising that effectors secreted via the T3SS are coregulated with the LEE, previous studies in EHEC and C. rodentium have not found these two genes to be regulated by Ler [34,45]. Only 4 extra-LEE genes were identified as part of the EHEC Ler regulon: sfdI, as discussed above; stcE, encoding a protease that is known to be involved in intimate adhesion and inhibition of complement-mediated lysis [32,46]; espC, located.

### Table 4. EPEC genes repressed 2-fold or more by Ler at late-log phase (OD_{600} = 0.9).

| Systematic Gene Name | Fold Repression | Common Gene Name | Gene Product | MGE |
|----------------------|----------------|------------------|--------------|-----|
| E2348C_0283          | 3              | yapU             | conserved inner membrane protein | ±IE1b |
| E2348C_1068          | 2              | ycdO             | hypothetical protein | |
| E2348C_1662          | 2              | ydfI             | predicted mannionate dehydrogenase | |
| E2348C_1664          | 3              | rpaB             | predicted oxidoreductase, Zn-dependent & NAD(P)-binding | |
| E2348C_1665          | 3              | rpaA             | predicted dehydratase | |
| E2348C_3384          | 2              | uxaA             | altronate hydrolase | |
| E2348C_3385          | 2              | uxaC             | uronate isomerase | |
| E2348C_3401          | 3              | yhoO             | predicted transporter | |
| E2348C_3751          | 2              | hdeB             | acid-resistance protein | |
| E2348C_3752          | 2              | hdeA             | stress response protein acid-resistance protein | |
| E2348C_4130          | 3              | metE             | 5-methyltetrahydropteroylglutamate-homocysteine S-methyltransferase | |
| E2348C_4624          | 9              | fimD             | outer membrane usher protein, type 1 fimbrial synthesis | |

Fold repression shows expression in ler/-ler cells. ±IE1b, directly adjacent to IE1b. For each gene reported, t-test P-value was less than 0.05.

doi:10.1371/journal.pone.0080160.t004
immediately downstream of stcE and encoding a component of the pO157-encoded type II secretion system for StcE is also known to be involved in adherence and intestinal colonization [47] and the putative cytochrome gene yhaI. Assuming that etpC is in the same operon as stcE, only the last of these is a novel observation.

We have also identified a number of EPEC genes that are repressed by Ler, including the ‘plasmid-encoded regulator’ operon perABC, located on the EPEC adherence factor (EAF) plasmid pMAR2 [48]. PerA protein activates transcription of the bfp operon, encoding bundle-forming pili [49]. These pili are involved in formation of an initial attachment between EPEC cells and the gut epithelium that occurs prior to AE lesion formation, therefore down-regulation of bfp expression with LEE expression is consistent with the known program of infection [50]. PerC protein is known to activate ler [17,18,51] and therefore this result suggests the existence of a negative feedback loop, previously undescribed, that ultimately autoregulates expression of Ler (and therefore the LEE) and may be involved in a down-regulation of ler transcription.

**Table 5.** EHEC genes activated 2-fold or more by Ler at late-log phase (OD600 = 1.1).

| Systematic Gene Name | Fold Activation | Common Gene Name | Gene Product | MGE   |
|----------------------|-----------------|------------------|--------------|-------|
| L7031                | 4               | stcE             | secreted zinc metalloprotease | pO157 |
| L7032                | 2               | etpC             | component of type II secretion system for StcE | pO157 |
| Z0955                | 5               | -                | systemic factor protein A homologue | PP (OI#36) |
| Z4458                | 36              | yhaI             | putative cytochrome |       |
| Z5100                | 7               | espF             | LEE-encoded effector EspF | LEE (OI#148) |
| Z5102                | 24              | orf29            | component of T3SS, SsaH family | LEE (OI#148) |
| Z5103                | 14              | escF             | T3SS structure protein EscF | LEE (OI#148) |
| Z5104                | 12              | -                | chaperone CesD2 | LEE (OI#148) |
| Z5105                | 9               | espB             | translocator EspB | LEE (OI#148) |
| Z5106                | 12              | espD             | translocator EspD | LEE (OI#148) |
| Z5107                | 24              | espA             | translocator EspA | LEE (OI#148) |
| Z5108                | 13              | sepL             | secretion switching protein SepL | LEE (OI#148) |
| Z5110                | 32              | eae              | intimin Eae | LEE (OI#148) |
| Z5111                | 30              | cesT             | chaperone CesT | LEE (OI#148) |
| Z5112                | 4               | tir               | translocated intimin receptor Tir | LEE (OI#148) |
| Z5113                | 12              | map              | LEE-encoded effector Map | LEE (OI#148) |
| Z5115                | 6               | espH             | LEE-encoded effector EspH | LEE (OI#148) |
| Z5116                | 5               | sepQ             | T3SS structure protein SepQ | LEE (OI#148) |
| Z5117                | 6               | orf16            | hypothetical protein | LEE (OI#148) |
| Z5118                | 6               | orf15            | hypothetical protein | LEE (OI#148) |
| Z5119                | 5               | escN             | translocator EscN | LEE (OI#148) |
| Z5120                | 6               | escV             | translocator EscV | LEE (OI#148) |
| Z5121                | 6               | mpc              | regulator Mpc | LEE (OI#148) |
| Z5122                | 6               | sepZ             | LEE encoded effector SepZ (EspZ) | LEE (OI#148) |
| Z5123                | 6               | rorf8            | chaperone Rorf8 | LEE (OI#148) |
| Z5124                | 7               | escJ             | T3SS structure protein EscJ | LEE (OI#148) |
| Z5125                | 8               | sepD             | secretion switching protein SepD | LEE (OI#148) |
| Z5126                | 7               | escC             | T3SS structure protein EscC | LEE (OI#148) |
| Z5127                | 6               | cesD             | chaperone CesD | LEE (OI#148) |
| Z5128                | 8               | grlA             | positive regulator GrlA | LEE (OI#148) |
| Z5129                | 4               | grlR             | negative regulator GrlR | LEE (OI#148) |
| Z5134                | 8               | escS             | T3SS structure protein EscS | LEE (OI#148) |
| Z5135                | 9               | escR             | T3SS structure protein EscR | LEE (OI#148) |
| Z5136                | 7               | orf5             | hypothetical protein | LEE (OI#148) |
| Z5137                | 6               | orf4             | hypothetical protein | LEE (OI#148) |
| Z5138                | 6               | orf3             | hypothetical protein | LEE (OI#148) |
| Z5139                | 7               | orf2             | hypothetical protein | LEE (OI#148) |
| Z5140                | 27              | ler               | transcription regulator Ler | LEE (OI#148) |
| Z5142                | 5               | espG             | LEE-encoded effector EspG | LEE (OI#148) |

Fold activation shows expression in ler ‘+’ ler cells. ±IE1b, directly adjacent to IE1b. For each gene reported, t-test P-value was less than 0.05. MGE, mobile genetic element; pO157, plasmid; PP, prophage; OI#, O-island number. For each gene reported, t-test P-value was less than 0.05.

doi:10.1371/journal.pone.0080160.t005
after the initial stages of infection [52]. Regulation of the per operon by Ler, the gene for which is known to be regulated by quorum sensing (QS), would account for the previously observed "indirect" QS regulation of perA [20]. The repressive effect of Ler on perA presumably also explains the up-regulation of the bundle-forming pilus (bfp) operon in the ler knockout mutant. Neither of these phenomena (which were only observed in mid-log phase cells) have so far been reported in the literature, although Elliot et al. reported Ler regulation of non-BFP fimbriae, while Leventon and Kaper described an inverse relationship between expression of ler and bfpA in the presence of HEp-2 cells [12,52]. Ler repression of acid resistance genes – previously noted by Abe et al. in the Sakai strain [36] - may reflect an accessory mechanism to assist in tight regulation of these genes, preventing inappropriate expression in the lower regions of the GI tract where acid resistance is not required.

Overall the data reported here suggests that the Ler regulon for enteropathogenic and enterohemorrhagic strains of E. coli is mainly focused on the type III secretion system genes in the LEE, but also includes unlinked pathogenicity genes. The regulon is growth phase dependent and, at least in strain E2348/69, is composed of both positively and negatively regulated genes. Additionally, in enteropathogenic E. coli, the observed negative regulation by Ler of PerC, itself a positive regulator of the ler operon, suggests the existence of a negative feedback loop involving these two proteins.

Materials and Methods

Bacterial strains and plasmids

Bacterial strains used or constructed during this study are detailed in table 6 and plasmids used or constructed are detailed in table 7. Standard techniques for recombinant DNA manipulations were used throughout this work. All cloned sequences were checked using the University of Birmingham Functional Genomics Facility (http://www.genomics.bham.ac.uk/sequencing.htm).

| Strain            | Description                                      | Reference                          |
|-------------------|--------------------------------------------------|------------------------------------|
| E. coli EDL933    | Enterohaemorrhagic E. coli Str- derivative        | Arthur Donohue- Rolfe, Tufts Cummings School of Veterinary Medicine; [59] |
| E. coli E2348/69  | Enteropathogenic E. coli                         | [60]                               |
| LBEC1             | EDL933 Δler                                      | This study                         |
| LBEC2             | E2348/69 Δler                                    | This study                         |

doi:10.1371/journal.pone.0080160.t006

Microarray Analysis of the Ler Regulon

Figure 1. Growth phase dependent Ler regulation of the EPEC LEE1 operon. While the other major operons of the EPEC LEE are regulated by Ler in a similar manner in both mid and late-log phase cultures, the LEE1 operon (ler - escU) was strongly activated by Ler only in late log phase cultures. The LEE1 operon and flanking genes are shown as block arrows and are coloured according to the fold activation seen in ler+ cells. Fold activation values are not shown for the ler gene as this is partly deleted in ler- cells. The intergenic region between ler and espG has been contracted for clarity.

doi:10.1371/journal.pone.0080160.g001
of all three plasmids (pDOC-derived donor plasmids and pACBSCE, pCP20) involved in mutagenesis was confirmed by antibiotic resistance profiling. The DNA sequence surrounding the recombination site was checked by sequencing across the knockout locus from primers designed to bind flanking sites. Recombinant strains were designated LBEC1 (EDL933 Δler) and LBEC2 (E2348/69 Δler).

The absence of gross unwanted deletions in the mutant strains was confirmed by comparative genomic hybridization (CGH) of labeled genomic DNA extracted from wild-type and mutant (Δler) strains. No missing loci, other than the desired deletion of ler, were apparent. Growth curves were assessed for LBEC1 and LBEC2 strains in comparison to parental wild-types and no gross defects in growth were observed (a small growth advantage consistent with predicted increased fitness due to reduced expression of T3SS was sometimes observed for the mutant strains on growth in inducing Dulbecco’s Modified Eagle Medium (DMEM) medium, but this was neither statistically significant nor reproducible).

The ler mutation in strains LBEC1 and LBEC2 was successfully complemented using the ler expression plasmid pSI04 resulting in the restoration of a functional T3SS, as confirmed by the fluorescent actin staining (FAS) test (i.e. via microscopic assessment of AE lesion formation (table 8) [58]. Subconfluent HeLa cell monolayers on glass coverslips were infected for 4 hours at 37 °C with a 1:10 dilution of an overnight LB broth culture of E. coli ler strains, grown in LB broth (Miller formulation) were diluted 1/100 into DMEM buffered with 25 mM HEPES and incubated at 37 °C, with aeration by shaking at 200 rpm (i.e. inducing conditions for expression of the LEE). Samples were harvested at mid and late log phases of growth (OD600 of 0.4 and 0.9 for EPEC; 0.5 and 1.1 for EHEC). Messenger RNA was stabilized immediately by pipetting the samples directly into RNAProtect Bacteria reagent (Qiagen) before purification of total RNA using the RNeasy Mini Kit with on-column DNase digestion (Qiagen).

**Microarray labelling and hybridization**

The concentration of RNA was determined using a spectrophotometer (ND-1000; NanoDrop). Five hundred nanograms of total RNA was used for labelling, and aRNA was synthesized with the Ambion MessageAmpTM II-Bacteria RNA Amplification Kit according to the recommendations of the manufacturer and labeled with the Cy3 or Cy5 monoreactive dye pack (GE Healthcare). Labeled aRNA was purified with Qiagen RNeasy MinElute clean up kit according to the manufacturer’s instructions and quantified using a spectrophotometer (ND-1000; NanoDrop). The 8×15,000 (15K) DNA high-density microarrays of E2348/69 and EDL933 were designed by Oxford Gene Technology (Oxford, UK) and validated by the University of Birmingham E. coli Centre (UBEC) (United Kingdom). During validation, three 60-mer probes per predicted gene were designed for all the open reading frames (ORFs) in the chromosome and plasmids of each one of the two E. coli strains used in this study. For each of the designed probes, a mismatch probe (containing 3 mismatches per 60-mer probe at positions 10, 25, and 40) was also generated. These mismatch probes and the perfect-match probes designed against each strain were placed on an array (4×44k) in triplicate. This array was hybridized with genomic DNA and a pool of mRNA representing conditions in which as many genes as practicable would be induced (derived from an equimolar pool of total RNA from E. coli grown in morpholinepropanesulfonic acid (MOPS) minimal medium at 30 °C mid-log phase, 37 °C for mid-log phase, and 37 °C for stationary phase). The results were processed to select the best-performing probe for each gene. This derived and optimized probe set was printed in a random pattern in triplicate with Agilent Technologies on an 8×15K array for each strain and used in this study. For each of the four biological replicates equal quantities (300 ng) of Cy5- and Cy3-labeled aRNA were added to hybridization solution, and hybridization was performed using the Gene Expression hybridization kit (Agilent Technologies).
Analysis of Microarray Data

The microarray images were analyzed using GenePix software version 6 (Axon Instruments). The data were imported into GeneSpring, version 7 (Agilent). A Lowess curve (locally weighted linear regression curve) was fitted to the plot of log intensity versus log ratio, and 40% of the data were used to calculate the Lowess fit at each point. The curve was used to adjust the control value for each measurement. If the control channel signal was below a threshold value of 10, then 10 was used instead.

For each strain data set a list of genes was prepared showing at least 2-fold differential expression levels between the lqf and wild type samples for each of the two growth conditions by using Student’s t-test and applying the Benjamini and Hochberg false discovery rate (multiple testing correction, MTC) test with a p value cut off of 0.05.

Acknowledgments

The authors would like to thank Dr.Y. Sevastsyanovich for commenting on a draft of this manuscript.

Author Contributions

Conceived and designed the experiments: LEHB CC LASS CWJ SVWB MSP. Performed the experiments: LEHB CC MP RKS MSI DJL. Analyzed the data: LEHB CC LASS. Contributed reagents/materials/analysis tools: MSP DJL. Wrote the paper: LEHB CC MSP RKS.

References

1. Kaper JB, Nataro JP, Mobley HL (2004) Pathogenic Escherichia coli. Nat Rev Microbiol 2: 125–140.
2. Oriol A, Greninger LA, McDaniel TK, Jarvis KG, Deng VK, et al. (1998) The complete sequence of the locus of enterocyte effacement (LEE) from enteropathogenic Escherichia coli E2348/69. Mol Microbiol 28: 1–4.
3. McDaniel TK, Jarvis KG, Domerger MS, Kaper JB (1995) A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. Proc Natl Acad Sci USA 92: 1664–1668.
4. Frankel G, Phillips AD, Rosenshine I, Dougan G, Kaper JB, et al. (1998) Enteropathogenic and enterohaemorrhagic Escherichia coli: more subversive elements. Mol Microbiol 30: 911–921.
5. Ogura Y, Ooka T, Iuchi A, Tsub T, Audulhamli M, et al. (2009) Comparative genomics reveal the mechanism of the parallel evolution of O157 and non-O157 enterohemorrhagic Escherichia coli. Proc Natl Acad Sci USA 106: 17939–17944.
6. Fohe T, Beaton SA, Taniguchi H, Abe H, Bailey CM, et al. (2006) An extensive repertoire of type III secretion effectors in Escherichia coli O157 and the role of lambdoid phages in their dissemination. Proc Natl Acad Sci USA 103: 14941–14946.
7. Wong ARC, Pearson JS, Bright MD, Munera D, Robinson KS, et al. (2011) Thermoregulated expression of transposon Tn1545 in E. coli strain CFT073. Microbiology 157: 458–472.
8. Spears KJ, Roe AJ, Gally DL (2006) A comparison of enteropathogenic and enterohemorrhagic Escherichia coli pathogenesis. FEMS Microbiol Lett 255: 187–202.
9. Vanaja SK, Springman AC, Besser TE, Whittam TS, Manning SD (2010) Differential expression of virulence and stress fitness genes between Escherichia coli O157:H7 strains with clinical or bovine-biased genotypes. Appl Environ Microbiol 76: 60–68.
10. Barba J, Bustamante VH, Flores-Valdez MA, Deng WY, Finlay BB, et al. (2005) A positive regulatory loop controls expression of the locus of enterocyte effacement-encoded regulator. Mol Microbiol 57: 1123–1133.
11. Islam MS, Bingle LEH, Pullen MJ, Busby JW (2011) Organization of the LEE operon in the region of enterohaemorrhagic Escherichia coli O157:H7 and activation by GrLA. FEMS Microbiol Lett 315: 135–136.
12. Porter ME, Mitchell P, Free A, Smith DGE, Gally DL (2005) The role of lambdoid phages in their dissemination. Proc Natl Acad Sci USA 103: 14941–14946.
13. Bustamante VH, Suntana FJ, Calva E, Puente JL (2001) Transcriptional regulation of the locus of enterocyte effacement-encoded regulators Ler and GrlA. J Bacteriol 183: 7918–7930.
14. Umanski T, Rosenshine I, Friedberg D (2002) A regulator of type III secretion genes in enteropathogenic Escherichia coli. J Biol Chem 277: 32336–32342.
15. Shuler SA, Kaper JB (2006) A new model for enterohemorrhagic Escherichia coli virulence gene regulation. Infect Immun 74: 4581–4586.
16. Flawed NL, Arora MS, Carmona AM (2007) Enteropathogenic and enterohemorrhagic Escherichia coli virulence gene regulation. Infect Immun 75: 4581–4586.
17. Sharma VK, Zuerner RL (2004) Role of lmrR family in the regulation of the esp operon in enterohemorrhagic Escherichia coli. Proc Natl Acad Sci USA 101: 14941–14946.
18. Kovalenko-Trotz S, Saldana Z, Deng W, Castaneda E, Freer E, et al. (2010) Bacterial macroscopic rope-like fibers with cytopathic and adhesive properties. J Biol Chem 285: 32336–32342.
19. Bergholdt TM, Wick LM, Qi W, Riorand JT, Ouellette LM, et al. (2007) Global transcriptional response of Escherichia coli O157:H7 to growth transitions in glucose minimal medium. BMC Microbiol 7: 97.
20. Gally DL (2006) A comparison of enteropathogenic and enterohemorrhagic Escherichia coli pathogenesis. FEMS Microbiol Lett 255: 187–202.
21. Porter ME, Mitchell P, Free A, Smith DGE, Gally DL (2005) The role of lambdoid phages in their dissemination. Proc Natl Acad Sci USA 103: 14941–14946.
22. Sperandio V, Li CC, Kaper JB (2002) Quorum-sensing Escherichia coli regulator A: a regulator of the LytR family involved in the regulation of the locus of enterocyte effacement pathogenicity island in enterohemorrhagic E. coli. Infect Immun 70: 3995–3997.
23. Umanski T, Rosenshine I, Friedberg D (2002) A regulator of type III secretion genes in enterohemorrhagic Escherichia coli. Microbiology 148: 2735–2744.
24. Saifima L, Valdez MA, Deng W, Finlay BB, et al. (2005) A positive regulatory loop controls expression of the locus of enterocyte effacement-encoded regulator. Mol Microbiol 57: 1123–1133.
25. Umanski T, Rosenshine I, Friedberg D (2002) A regulator of type III secretion genes in enterohemorrhagic Escherichia coli. Microbiology 148: 2735–2744.
26. Sperandio V, Li CC, Kaper JB (2002) Quorum-sensing Escherichia coli regulator A: a regulator of the LytR family involved in the regulation of the locus of enterocyte effacement pathogenicity island in enterohemorrhagic E. coli. Infect Immun 70: 3995–3997.
27. Sperandio V, Li CC, Kaper JB (2002) Quorum-sensing Escherichia coli regulator A: a regulator of the LytR family involved in the regulation of the locus of enterocyte effacement pathogenicity island in enterohemorrhagic E. coli. Infect Immun 70: 3995–3997.
28. Sperandio V, Li CC, Kaper JB (2002) Quorum-sensing Escherichia coli regulator A: a regulator of the LytR family involved in the regulation of the locus of enterocyte effacement pathogenicity island in enterohemorrhagic E. coli. Infect Immun 70: 3995–3997.
29. Sperandio V, Li CC, Kaper JB (2002) Quorum-sensing Escherichia coli regulator A: a regulator of the LytR family involved in the regulation of the locus of enterocyte effacement pathogenicity island in enterohemorrhagic E. coli. Infect Immun 70: 3995–3997.
40. Navarre WW, Porwollik S, Wang Y, McClelland M, Rosen H, et al. (2006) Selective silencing of foreign DNA with low GC content by the H-NS protein in Salmonella. Science 313: 236–238.

41. Bishop RE, Kim S-H, El Zoeiby A (2005) Role of lipid A palmitoylation in bacterial pathogenesis. J Endotox Res 11: 174–180.

42. Mildner-Earley S, Miller VL (2006) Characterization of a novel porin involved in systemic Erwinia uredovora infection. Infect Immun 74: 4361–4365.

43. Keenleyside WJ, Jayaratne P, MacLachlan PR, Whitfield C (1992) The rcsA gene of Escherichia coli O9:K30:H12 is involved in the expression of the serotype-specific group I K (capsular) antigen. J Bacteriol 174: 8–16.

44. Mellies JL, Barron AM, Haack KR, Korson AS, Oldridge DA (2006) The global regulator Ler is necessary for enteropathogenic Escherichia coli colonization of Caenorhabditis elegans. Infect Immun 74: 64–72.

45. Garcia-Angulo VA, Deng W, Thomas NA, Finlay BB, Puente JL (2008) Regulation of expression and secretion of NleH, a new locus of enterocyte effacement-encoded effector in Citrobacter rodentium. J Bacteriol 190: 2388–2399.

46. Lathem WW, Bergsbaken T, Welch RA (2004) Potentiation of C1 esterase inhibitor by StcE, a metalloprotease secreted by Escherichia coli O157:H7. J Exp Med 199: 1077–1087.

47. Ho TD, Davis BM, Ritchie JM, Waldor MK (2008) Type 2 secretion promotes enterohemorrhagic Escherichia coli adherence and intestinal colonization. Infect Immun 76: 1034–1043.

48. Gomez-Duarte OG, Kaper JB (1995) A plasmid-encoded regulatory region activates chromosomal eaeA expression in enteropathogenic Escherichia coli. Infect Immun 63: 1767–1776.

49. Toke T, Schoolnik GK, Sohel I, Bustamante VH, Puente JL (1996) Cloning and characterization of bfpA, genes required for the transcriptional activation of bfpA in enteropathogenic Escherichia coli. Mol Microbiol 21: 963–975.

50. Cleary J, Lai L-C, Shaw RK, Straatman-Iwanowska A, Donnenberg MS et al. (2004) Enteropathogenic Escherichia coli (EPEC) adhesion to intestinal epithelial cells: role of bundle-forming pili (BFP), EspA filaments and intimin. Microbiology 150: 527–538.

51. Bustamante VH, Villalba MI, Garcia-Angulo VA, Vazquez A, Martinez LC, et al. (2011) PerC and GrA independently regulate Ler expression in enteropathogenic Escherichia coli. Mol Microbiol 82: 398–415.

52. Levertov LQ, Kaper JB (2005) Temporal expression of enteropathogenic Escherichia coli virulence genes in an in vitro model of infection. Infect Immun 73: 1034–1043.

53. Wade JT, Belyaeva TA, Hyde EL, Busby SJW (2000) Repression of the Escherichia coli melR promoter by MelR: evidence that efficient repression requires the formation of a repression loop. Mol Microbiol 36: 223–229.

54. Islam MS (2011) Studies on gene expression in a pathogenic bacterium. PhD Thesis. University of Birmingham, UK.

55. Lee DJ, Bingle LEH, Heurlier K, Pallen MJ, Penn CW, et al. (2009) Gene expression in enteropathogenic Escherichia coli strains. BMC Microbiol 9: 252.

56. Cherepanov PP, Wackernagel W (1995) Gene disruption in Escherichia coli: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic resistance determinant. Gene 158: 9–14.

57. Mellies JL, Larabee FJ, Zarr MA, Hoekbak KL, Lorenzen E, et al. (2006) Ler interdomain linker is essential for anti-silencing activity in enteropathogenic Escherichia coli. Microbiology 154: 3624–3638.

58. Knutton S, Baldwin T, Williams PH, McNeish AS (1989) Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic Escherichia coli. Infect Immun 57: 1290–1296.

59. Perna NT, Plunkett G, Burland V, Glasner JD, et al. (2001) Genome sequence of enterohaemorrhagic Escherichia coli O157:H7. Nature 409: 529–533.

60. Iguchi A, Thomson NR, Ogura Y, Saunders D, Osuka T, et al. (2009) Complete genome sequence and comparative genome analysis of enteropathogenic Escherichia coli O127:H6 strain E2348/69. J Bacteriol 191: 347–354.