Escherichia coli type III secretion system 2: a new kind of T3SS?

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Abstract

Type III secretion systems (T3SSs) are employed by Gram-negative bacteria to deliver effector proteins into the cytoplasm of infected host cells. Enteropathogenic Escherichia coli use a T3SS to deliver effector proteins that result in the creation of the attaching and effacing lesions. The genome sequence of the Escherichia coli pathotype O157:H7 revealed the existence of a gene cluster encoding components of a second type III secretion system, the E. coli type III secretion system 2 (ETT2). Researchers have revealed that, although ETT2 may not be a functional secretion system in most (or all) strains, it still plays an important role in bacterial virulence. This article summarizes current knowledge regarding the E. coli ETT2, including its genetic characteristics, prevalence, function, association with virulence, and prospects for future work.

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1. Introduction

Many pathogenic Gram-negative bacteria utilize type III secretion systems (T3SSs) to subvert eukaryotic signaling pathways by injecting virulence proteins into the host cell cytoplasm [1-5]. Intracellular pathogens, such as Salmonella, Shigella, and Chlamydia, use T3SS for attachment to and/or invasion of host cells [6-8]. Yersinia enterocolitica and Yersinia pseudotuberculosis induce macrophage apoptosis and subvert host innate immunity by injecting effectors through the T3SS [9-11]. Enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC) use the T3SS to deliver effector proteins that result in the creation of the attaching and effacing (A/E) lesions [12,13]. Salmonella utilize multiple type III secretion systems, with the first, Salmonella pathogenicity islands 1 (SPI-1) T3SS, playing a role in invasion into host cells, while the second, SPI-2 T3SS, is important for intracellular survival [14]. The complete genome sequencing of two EHEC O157:H7 strains, EDL933 and Sakai, revealed the presence of a gene cluster predicted to encode an additional T3SS. This T3SS was designated as the E. coli type III secretion system 2 (ETT2), to distinguish it from the locus of enterocyte effacement (LEE) - encoded T3SS, which is now called ETT1 [15,16].

EHEC and other Shiga-like toxin-producing E. coli (STEC), including the O157:H7 serotype, are responsible for diseases in humans and animals whose clinical spectrum includes diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) [17-20]. There are no proven effective treatments, and administering antibiotics is often contraindicated because they may enhance the progression of enteritis to HUS [21]. While the function of the EHEC ETT1 has been well characterized [22-25], the function of ETT2 and its role in virulence is less clear.

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2. Genetic characteristics and differentiation of ETT2

The genes required for ETT1 function, including the T3SS apparatus and the secreted proteins, are encoded on the LEE in EPEC and EHEC. This virulence locus is about 35.4-kb in size and contains 41 open reading frames (ORFs) [24-26]. Up to 32 other non-LEE-encoded effector proteins can also be delivered into host cells by the ETT1 to subvert eukaryotic cell biology [27]. The ETT2 is approximately 29.9-kb in size and is adjacent to the tRNA locus glyU on the bacterial chromosome (Figure 1). The ETT2 gene cluster includes at least 35 ORFs, from ECs3703 to ECs3737 in the Sakai strain (GenBank: NC_002695; Table 1). The G + C content of the ETT2 sequence is lower (36.9%) than that of the E. coli chromosome (50.8%), suggesting that the ETT2 gene cluster was a pathogenicity island inserted into EHEC O157:H7 strains [28,29].

It has been shown that the ETT2 gene cluster exists in whole or in part in the majority of E. coli strains, regardless of their pathogenicity, but with significant variability [29-31]. Sequence alignment analysis revealed that the ETT2 with all 35 ORFs in the Sakai strain also contains some pseudogenes, while EAEC 042 not (Figure 1, [31]). Cheng divided ETT2 variants into 11 isoforms (designated as type A to type K), with type A representing the complete ETT2 island with 35 ORFs [32].

3. Prevalence and distribution of ETT2

The ETT2 gene cluster is primarily associated with STEC strains. Hartleib et al. detected the presence of ETT2 locus in 245 strains of E. coli, in 25 strains of Salmonella enterica, and in 10 other bacteria, using both PCR and Southern blotting. The ETT2 was distributed ubiquitously among intestinal E. coli strains, but not in extra-intestinal (including serotypes O2:H7, O6:H12, O18:H7) or non-pathogenic (including serotypes O5, O74) E. coli strains or in other enteric bacteria such as Salmonella, Yersinia, and Shigella [28]. Serotype O55:H7 strains were shown to be the most recent precursors of the virulent O157:H7 EHEC strains [33]. BLAST Genome on NCBI revealed O55:H7 EPEC strains share 49% fragment of the intact ETT2 cluster, losing almost all genes from ECs3711 to ECs3731 (Table 1). Genome analysis also showed that, all whole-genome sequenced EPEC strains (except serotype O127:H6) and STEC (including serotypes both O157 and non-O157) strains carry the ETT2 gene cluster.

ETT2 is prevalent in a majority of characterized human diarrheagenic E. coli isolates (54/83; 65%), and all STEC strains tested (except serotype O177) contain the ETT2 gene cluster [34]. However, ETT2 prevalence in uropathogenic E. coli (UPEC) strains is low [35].

In animal originated E. coli strains, ETT2 is also common. Cheng et al. isolated 92 pathogenic E. coli strains from weaned piglets with edema and/or diarrhea and 76 pathogenic E. coli strains from dairy cows with clinical or sub-clinical mastitis and detected the ETT2 ORFs by PCR. Most isolates (85.9%) from piglets contained an intact or partial ETT2 gene cluster, significantly higher than those isolated from cows (47.4%) [32]. Prager also identified ETT2 loci in 117 STEC strains originating from piglets suffering from edema disease or colibacillosis [30].

4. Putative functions of the ETT2

4.1 ETT2 encodes a functional secretion system?

The T3SSs are comprised of a basal body that spans the cell membranes and a needle-like complex that extends from the outer membrane, through which effectors are secreted. The basal body consists of an inner ring, an outer ring, and a neck that spans the periplasm [36]. The ETT2 gene cluster was originally regarded as a SPI-1-like island, of which 19 ORFs are highly homologous with the SPI-1 T3SS of Salmonella enterica serovar Typhimurium [37,38]. However, some significant differences exist between the E. coli ETT2 and SPI-1 T3SS:
Table 1 Homologs and putative functions of ETT2 ORFs

| Gene in Sakai strain | Length (bp) | Homologs* | Putative function |
|----------------------|-------------|-----------|------------------|
| ECs3703              | 693         | yqeH      | Sensory transducer |
| ECs3704              | 810         | yqeI      | Sensory transducer |
| ECs3705              | 480         | yqeJ      | Sensory transducer |
| ECs3706              | 438         | yqeK      | Sensory transducer |
| ECs3707              | 492         | yqeF      | Sensory transducer |
| ECs3708              | 492         | ygeG, sicA| Chaperone |
| ECs3709              | 1377        | yqeH      | Transcriptional regulator |
| ECs3710              | 219         | B2853     | Inner membrane protein |
| ECs3711              | 402         | B2854, iagB| Lipoprotein precursor |
| ECs3712              | 633         | ygeK/B2856, ssrB| Surface presentation of antigens protein |
| ECs3713              | 432         | B2857     | Inner membrane protein |
| ECs3714              | 261         | B2858, orgB| Transcriptional regulator |
| ECs3715              | 582         | B2859, orgA| Transcriptional regulator |
| ECs3716/eprK         | 735         | pkgK      | Lipoprotein precursor |
| ECs3717/eprJ         | 333         | pkgl, mxl| Transcriptional regulator |
| ECs3718/eprP         | 240         | pkgl, mxH| Transcriptional regulator |
| ECs3719/eprY         | 735         | pkgH, mxG| Transcriptional regulator |
| ECs3720/etra         | 501         | ntrC      | Transcriptional regulator |
| ECs3721/epaS         | 1122        | spaS      | Surface presentation of antigens protein |
| ECs3722/epaR2        | 237         | spaR      | Surface presentation of antigens protein |
| ECs3723/epaR1        | 468         | spaQ      | Surface presentation of antigens protein |
| ECs3724/epaQ         | 261         | spaQ      | Surface presentation of antigens protein |
| ECs3725/epaP         | 666         | spaP      | Surface presentation of antigens protein |
| ECs3726/epaO         | 987         | spaO      | Surface presentation of antigens protein |
| ECs3727/eivJ         | 618         | spaH/invJ| Invasion protein |
| ECs3728              | 234         |           |                  |
| ECs3729/eivI         | 336         | spaM/invI| Invasion protein |
| ECs3730/eivC         | 1320        | spa/invC  | Invasion protein |
| ECs3731/eivA         | 2061        | invA      | Invasion protein |
| ECs3732/eivE         | 1146        | invE      | Invasion protein |
| ECs3733/eivG         | 1704        | invG      | Invasion protein |
| ECs3734/eivF         | 750         | invF      | Transcriptional regulator |
| ECs3735              | 180         | ydcX      | Inner membrane protein |
| ECs3736/pkgA         | 1059        | B2863     | Phosphorylase kinase and glucosylase |
| ECs3737              | 606         | B2862     | Phosphorylase kinase and glucosylase |

*yeHTK, yqeFGH, ydcX, B2853, B2354, B2356-B2359, B2362, and B2363 are conserved genes in E. coli K-12. orgA, orgB, lalgB, prgHKU, spaOQRS, invA, invC, invF, invV, invG, invI, and invJ are Salmonella enterica SPI-1 genes. mxl, mxG and mxH are Shigella flexneri genes. ntrC is from Herbaspiillum seropedicae.
the meningitis-causing *E. coli* K1 strain EC10 and observed that the ETT2 deletion mutant exhibited both a 50% reduction in invasion and an 80% decrease in intracellular survival in human brain microvascular endothelial cells (HBMECs) as compared with WT EC10 [48], which indicated that ETT2 is necessary in the pathogenic process of *E. coli* strain K1 interacting with host cells. Sheikh et al. also reported that a hilA (encoding a *Salmonella enterica* regulator) homolog, eilA, coordinately activated both the EivF, EivA and presumably other effectors encoded elsewhere on the genome (adjacent to tRNA locus selC), influencing both bacterial adherence to epithelial cells and biofilm formation, which indirectly suggested the role of ETT2 in bacterial virulence [49,50].

### 4.3 Association of ETT2 with other virulence factors

In the veterinary field, as the ETT2 is primarily associated with EHEC and STEC, it is common to find Stx2e in ETT2 positive strains [32]. In other pathogenic *E. coli* of different O: H serotypes, the ETT2 island is also associated with one or more pathogenicity islands, such as LEE, HPI and LPA [28].

Prager et al. investigated 266 STEC isolates from various human clinical sources and found that different virulence genes were associated with the ETT2. Some genes, including stx2d (encoding a Shiga toxin activated by elastase) occur alone or in combinations with ETT2 or LPA, but never with the LEE [51]. It will be important to confirm whether the expression and/or secretion of these virulence factors are affected by the ETT2 island.

### 5. Conclusions

The ETT2 is prevalent in pathogenic EHEC and STEC strains and is likely important to both human and veterinary medicine. While the exact function of the ETT2 has not been defined clearly, rather than encoding a secretion system, recent work has demonstrated its importance to bacterial adherence and in regulating the expression of other virulence factors, and further study of the ETT2 may help to elucidate details governing bacterial virulence gene regulation. While most serotypes of *E. coli* carry an impaired ETT2 gene cluster, the presence of an intact ETT2 might be used to identify highly-pathogenic EHEC or STEC strains and for molecular fingerprinting of epidemic strains in humans and animals.

### 6. Competing interests

The authors declare that they have no competing interests.

### 7. Authors’ contributions

MZ and ZG collected the data. MZ reviewed the collections and wrote the manuscript. GZ, PRH and QD gave their comments and helped revising this manuscript. All authors read and approved the final manuscript.

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### 9. References

1. Hueck CJ: Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol Mol Biol Rev* 1998, 62:729–433.
2. Kenny B: Mechanism of action of EPEC type III effector molecules. *Int J Med Microbiol* 2002, 291:469–477.
3. Lee CA: Type III secretion systems: machines to deliver bacterial proteins into eukaryotic cells? Trends Microbiol 1997, 5:148–156.
4. Cornelis GR: *Yersinia* type III secretion: send in the effectors. *J Cell Biol* 2002, 158:401–408.
5. Rao X, Beatty WE, Fan H: Exploration of chlamydial type III secretion system reconstitution in *Escherichia coli*. *PLoS One* 2012, 7:e50833.
6. Ménard R, Dehio C, Sansonetti PJ: Bacterial entry into epithelial cells: the paradigm of *Shigella*. *Trends Microbiol* 1996, 4:220–226.
7. Galan JE: *Salmonella* interactions with host cells: type III secretion at work. *Annu Rev Cell Dev Biol* 2001, 17:53–86.
8. Fields KA, Mead DJ, Dooley CA, Hackstadt T: *Chlamydia trachomatis* type III secretion: evidence for a functional apparatus during early-cycle development. *Mol Microbiol* 2003, 48:671–683.
9. Mills SD, Boland A, Sory MP, van der Simmen P, Kerbourch C, Finlay BB, Cornelis GR: *Yersinia enterocolitica* induces apoptosis in macrophages by a process requiring functional type III secretion and translocation mechanisms and involving YopP, presumably acting as an effector protein. *Proc Natl Acad Sci U S A* 1997, 94:12628–12633.
10. Momack DM, Mecsas J, Ghori N, Falkow S: *Yersinia* signals macrophages to undergo apoptosis and YopJ is necessary for this cell death. *Proc Natl Acad Sci U S A* 1997, 94:10385–10390.
11. Bliska JB, Wang X, Viboud C, Brodsky E: Modulation of innate immune responses by *Yersinia* type III secretion system translocators and effectors. *Cell Microbiol* 2013, 15:1622–1631.
12. Celli J, Deng W, Finlay BB: Entero-pathogenic *Escherichia coli* (EPEC) attachment to epithelial cells: exploiting the host cell cytoskeleton from the outside. *Cell Microbiol* 2000, 2:1–9.
13. Janiv KG, Kaper JB: Secretion of extracellular proteins by enterohemorrhagic *Escherichia coli* via a putative type III secretion system. *Infect Immun* 1996, 64:4826–4829.
14. Silva-Herzog E, Dettweiler CS, Salmonella enterica replication in homophasic macrophages requires two type three secretion systems. *Infect Immun* 2010, 78:3369–3377.
15. Hayashi T, Makino K, Ohtsubo E, Nakayama K, Murata T, Tanaka M, Tober T, Iida T, Takami H, Honda T, Sasaki C, Ogawara N, Yasunaga T, Kuhara S, Shibata S, Hatton M, Shinagawa H: Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. *DNA Res* 2001, 8:11–22.
16. Perkins NT, Plunkett G 3rd, Burland V, Mau B, Glasner JD, Rose DJ, Mayhew GF, Evans PS, Gregor J, Kirkpatrick HA, Pösfai G, Hackett J, Klink S, Boutin A, Shao Y, Miller L, Grotbeck EJ, Davis NW, Lin A, Dimanlata ET, Potamousis KD, Apodaca J, Anantharaman TS, Lin J, Yen G, Schwartz DC, Welch RA, Blattner FR, Genome
sequence of enterohaemorrhagic Escherichia coli O157:H7. Nature 2001, 409:59–53.

17. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML: Hemorrhagic colitis associated with a rare Escherichia coli serotype. N Engl J Med 1983, 308:681–685.

18. Cohen MB, Giannella RA: Hemorrhagic colitis associated with Escherichia coli O157:H7. Adv Intern Med 1992, 37:131–195.

19. Watanabe H, Wada A, Inagaki Y, Itoh K, Tamura K: Outbreaks of enterohaemorrhagic Escherichia coli O157:H7 infection by two different genotype strains in Japan, 1996. Lancet 1996, 348:831–832.

20. Rivas M, Milliewsky E, Chinen I, Roldan CD, Balbi L, Garcia B, Fiorilli G, Sosa-Estani S, Kincard J, Rangel J, Griffin PM: Case–control Study Group: Characterization and epidemiologic subtyping of Shiga toxin-producing Escherichia coli strains isolated from hemolytic uremic syndrome and diarrhea cases in Argentina. Foodborne Pathog Dis 2006, 3:88–96.

21. Tzponi S, Sheoran A, Akiyoshi D, Donohue-Rollie A, Trachtman H: Antibody therapy in the management of shiga toxin-induced hemolytic uremic syndrome. Clin Microbiol Rev 2004, 17:926–941.

22. Pham TH, Gao X, Tsai K, Olsen R, Wan F, Hardwidge PR: Functional differences and interactions between the Escherichia coli type III secretion system effectors NleH1 and NleH2. Infect Immun 2012, 80:2133–2140.

23. Abe H, Miyahara A, Oshima T, Tashiro K, Ogura Y, Kuhara S, Ogasawara N, Hayashi T, Tobe T: Global regulation by horizontally transferred regulators establishes the pathogenicity of Escherichia coli. DNA Res 2008, 15:25–38.

24. Elliott SJ, Wainwright LA, McDaniel TK, Jarvis KG, Deng YK, Lai LC, McNamara BP, Dornemann MS, Kaper JB: The complete sequence of the locus of enterocyte effacement (LEE) from enteropathogenic Escherichia coli E2348/69. Mol Microbiol 1998, 28:1–4.

25. Gauthier A, Puente JL, Finlay BB: Secretion of the enteropathogenic Escherichia coli type III secretion system requires components of the type II apparatus for assembly and localization. Infect Immun 2003, 71:3310–3319.

26. McDaniel TK, Kaper JB: A cloned pathogenicity island from enteropathogenic Escherichia coli confers the attaching and effacing phenotype on E. coli K-12. Mol Microbiol 1997, 23:599–407.

27. Tobe T, Beaton SA, Taniguchi H, Abe H, Bailey CM, Fivian A, Younis R, Matthews S, Marches O, Frankel G, Hayashi T, Pallen MJ: 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 U S A 2006, 103:14941–14946.

28. Hardt WD, Ulfah B, Galian JE: A substrate of the centrosome 63 type III protein secretion system of Salmonella typhimurium is encoded by a cryptic bacteriophage. Proc Natl Acad Sci U S A 1998, 95:2574–2579.

29. Norris FA, Wilson MP, Wailes TS, Galyov EE, Majerus PW, Sobr, a protein required for virulence of Salmonella dublin, is an inositol phosphate phosphatase. Proc Natl Acad Sci U S A 1998, 95:14057–14059.

30. Zhang L, Chaudhuri RR, Constantindou C, Hobman JL, Patel MD, Jones AC, Sarti D, Roe AJ, Wåsdou I, Shaw RK, Falciari F, Stevens NP, Galy DL, Knutton S, Frankel G, Penn CW, Pallen MJ: Regulators encoded in the Escherichia coli type III secretion system 2 gene cluster influence expression of genes within the locus for enterocyte effacement in enterohemorrhagic E. coli O157:H7. Infect Immun 2004, 72:7282–7293.

31. Iredes D, Gophna U, Paitan Y, Chaudhuri RR, Pallen MJ, Ron EZ: A degenerate type III secretion system from septicemic Escherichia coli contributes to pathogenesis. J Bacteriol 2005, 187:8164–8171.

32. Yao Y, Xie Y, Perace D, Zhong Y, Lu J, Tao J, Guo X, Kim KS: The type III secretion system is involved in the invasion and intracellular survival of Escherichia coli K1 in human brain microvascular endothelial cells. FEMS Microbiol Lett 2009, 300:18–24.

33. Sheikh J, Dudley EG, Sui B, Tamboura B, Suleman A, Nataro JP, Eila, a HII-like regulator in enteropathogenic Escherichia coli. Mol Microbiol 2006, 61:338–350.

34. Kaper JB, Melles JL, Nataro JP: Pathogenicity islands and other mobile genetic elements of diarrheagenic Escherichia coli. In Pathogenicity islands and other mobile virulence elements. Washington, DC: American Society for Microbiology; 1999:33–58.

35. Prager R, Annemuller S, Tschape H: Diversity of virulence patterns among shiga toxin-producing Escherichia coli from human clinical cases-need for more detailed diagnostics. Int J Med Microbiol 2005, 295:29–38.

36. Comella GR: The type III secretion injectione. Nat Rev Microbiol 2006, 4:811–825.

37. Hansen-Wester I, Hensei M: Salmonella pathogenicity islands encoding type III secretion systems. Microbes Infect 2001, 3:549–559.

38. Laxhith CP, Lee CA: The Salmonella pathogenicity island-1 type III secretion system. Microbes Infect 2001, 3:1291–1291.

39. Ehrbar K, Friesel A, Miller SI, Hardt WD: Role of the Salmonella pathogenicity island 1 (SPI-1) protein InvB in type III secretion of SopE and SopE2, two Salmonella effector proteins encoded outside of SPI-1. J Bacteriol 2003, 185:9650–9667.

40. Daeffer S, Russel M: The Salmonella typhimurium InvH protein is an outer membrane lipoprotein required for the proper localization of InvG. Mol Microbiol 1998, 28:1367–1380.

41. Moest TP, Meresse S: Salmonella T3SS3: successful mission of the secretion (in) agents. Curr Opin Microbiol 2013, 16:38–44.

42. Pallen MJ: Glucoamylyse-like domains in the alpha- and beta-subunits of phosphorylase kinase. Protein Sci 2003, 12:1804–1807.

43. Chaudhuri RR, Sebahia M, Hobman JL, Webber MA, Leyton DL, Goldberg MD, Cunningham AF, Scott-Tucker A, Ferguson PR, Thomas CM, Frankel G, Tang CM, Dudley EG, Roberts B, Rasko DA, Pallen MJ, Parkhill J, Nataro JP, Thomson NR, Henderson IR: Complete genome sequence and comparative metabolic profiling of the prototypical enteropathogenic Escherichia coli strain 042. PLoS One 2010, 5:e8801.

44. Hardt WD, Ulfah B, Galian JE: A substrate of the centrosome 63 type III protein secretion system of Salmonella typhimurium is encoded by a cryptic bacteriophage. Proc Natl Acad Sci U S A 1998, 95:2574–2579.

45. Prager R, Annemuller S, Tschape H: Identification of the ET2 locus in human diarrheagenic Escherichia coli by multiplex PCR. J Infect Chemother 2006, 12:157–159.

46. Miyazaki J, Ba-Thein W, Kuma T, Akaza H, Hayashi H: Identification of a type III secretion system in uropathogenic Escherichia coli. FEMS Microbiol Lett 2003, 212:221–228.