



Widespread Strain-Specific Distinctions in Chromosomal Binding Dynamics of a Highly Conserved *Escherichia coli* Transcription Factor

 James P. R. Connolly,^a Nicky O'Boyle,^b  Andrew J. Roe^b

^aNU Biosciences Institute, Newcastle University, Newcastle-upon-Tyne, United Kingdom

^bInstitute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom

ABSTRACT Bacterial gene regulation is governed by often hundreds of transcription factors (TFs) that bind directly to targets on the chromosome. Global studies of TFs usually make assumptions that regulatory targets within model strains will be conserved between members of the same species harboring common genetic targets. We recently discovered that YhaJ of *Escherichia coli* is crucial for virulence in two different pathotypes but binds to distinct regions of their genomes and regulates no common genes. This surprising result leads to strain-specific mechanisms of virulence regulation, but the implications for other *E. coli* pathotypes or commensals were unclear. Here, we report that heterogenous binding of YhaJ is widespread within the *E. coli* species. We analyzed the global YhaJ binding dynamics of four evolutionarily distinct *E. coli* isolates under two conditions, revealing 78 significant sites on the core genome as well as horizontally acquired loci. Condition-dependent dosage of YhaJ correlated with the number of occupied sites *in vivo* but did not significantly alter its enrichment at regions bound in both conditions, explaining the availability of this TF to occupy accessory sites in response to the environment. Strikingly, only ~15% of YhaJ binding sites were common to all strains. Furthermore, differences in enrichment of uncommon sites were observed largely in chromosomal regions found in all strains and not explained exclusively by binding to strain-specific horizontally acquired elements or mutations in the DNA binding sequence. This observation suggests that intraspecies distinctions in TF binding dynamics are a widespread phenomenon and represent strain-specific gene regulatory potential.

IMPORTANCE In bacterial cells, hundreds of transcription factors coordinate gene regulation and thus are a major driver of cellular processes. However, the immense diversity in bacterial genome structure and content makes deciphering regulatory networks challenging. This is particularly apparent for the model organism *Escherichia coli* as evolution has driven the emergence of species members with highly distinct genomes, which occupy extremely different niches in nature. While it is well-known that transcription factors must integrate horizontally acquired DNA into the regulatory network of the cell, the extent of regulatory diversity beyond single model strains is unclear. We have explored this concept in four evolutionarily distinct *E. coli* strains and show that a highly conserved transcription factor displays unprecedented diversity in chromosomal binding sites. Importantly, this diversity is not restricted to strain-specific DNA or mutation in binding sites. This observation suggests that strain-specific regulatory networks are potentially widespread within individual bacterial species.

KEYWORDS transcription factor, regulation, *E. coli*, ChIP-seq, transcription factor

Citation Connolly JPR, O'Boyle N, Roe AJ. 2020. Widespread strain-specific distinctions in chromosomal binding dynamics of a highly conserved *Escherichia coli* transcription factor. mBio 11:e01058-20. <https://doi.org/10.1128/mBio.01058-20>.

Editor Vanessa Sperandio, University of Texas Southwestern Medical Center Dallas

Copyright © 2020 Connolly et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to James P. R. Connolly, James.Connolly2@newcastle.ac.uk, or Andrew J. Roe, Andrew.Roe@glasgow.ac.uk.

Received 24 April 2020

Accepted 27 May 2020

Published 23 June 2020

Gene regulation is at the core of all cellular processes, and its tailoring can drive new phenotypes that benefit bacterial cells (1, 2). Bacterial species carry genes that encode hundreds of transcription factors (TFs) that coordinate gene regulation, often in response to environmental stimuli (3–5). This process has been well studied for pathogens, as virulence factors are usually encoded on horizontally acquired genetic elements that require integration into the regulatory network of the cell. Variation in genomic content extends far beyond genes encoding virulence factors and while diversity in regulatory networks is well accepted for TF orthologues present in different species, the possibility that TFs can be tailored to individual members of the same species is largely unexplored (5, 6). Regulatory networks are often studied in *Escherichia coli* as a model organism (usually the nonpathogenic commensal K-12), but the vast genomic diversity within this species results in ecologically distinct strains that occupy extremely different niches (7–11). This is particularly prominent in pathotypes such as enterohemorrhagic *E. coli* (EHEC), uropathogenic *E. coli* (UPEC) and neonatal-meningitis *E. coli* (NMEC) that thrive in the terminal colon, urinary tract, and brain, respectively (12). The highly specific mechanisms that drive pathogenesis, as well as basic survival, in such distinct environments require gene regulation to be controlled on an individual level.

We recently discovered that a highly conserved *E. coli* LysR-type TF (named YhaJ) has been repurposed to perform drastically different roles in EHEC and UPEC (13, 14). YhaJ was found to regulate no common genes but activated virulence factors unique to each strain (type 3 secretion in EHEC and type 1 fimbriae in UPEC). We also observed distinctions in binding to conserved chromosomal targets (most strikingly the acid tolerance regulator *gadX*) and their subsequent regulation, but the reasons driving this were unknown. We noticed that YhaJ expression was dramatically higher in EHEC compared to UPEC when grown under identical conditions and hypothesized that this was a driver of the strain-specific gene regulation observed. This prompted us to examine the phenomenon using the divergent *yhaJ-yhaK* regulatory region as a model system. This region contains a YhaJ binding site and overlapping promoters that are 100% conserved in four evolutionarily distinct *E. coli* strains—EHEC, UPEC, NMEC, and K-12 (Fig. 1A and B). Note that the protein-coding sequence of YhaJ is completely identical except for an amino acid substitution in UPEC, which we previously confirmed does not impact its apparent functionality (14). Despite this commonality, testing YhaJ expression revealed that YhaJ dosage varied drastically between strains grown in minimal essential medium (MEM), with UPEC for example displaying significantly ($P = 0.036$) lower YhaJ expression than EHEC. In contrast, growth in rich media (LB) yielded almost identical expression levels of YhaJ in all strains (Fig. 1C). The phenomenon of TF dosage can impact specific stress responses and even offer an evolutionary advantage for individual strains, as has been described for the *E. coli* sigma factor RpoS (15–17). We reasoned that the natural variation in TF expression would correlate with binding levels to a common target. Surprisingly, chromatin immunoprecipitation (ChIP)-PCR analysis revealed that YhaJ enrichment at the *yhaK* promoter region did not vary with TF dosage. This was particularly prominent for UPEC in minimal medium, which displayed the highest enrichment of YhaJ signal at this region despite YhaJ expression being comparably lower (Fig. 1D). This result was corroborated by finding that naturally enhancing YhaJ expression levels in LB had no significant effect on YhaJ enrichment at this binding site. To confirm this phenomenon, we analyzed a known YhaJ target gene, *yqjF*, and similarly found that occupancy was not conditionally dependent or driven by YhaJ expression (see Fig. S1 in the supplemental material) (18). These results collectively indicate that differences in YhaJ enrichment at conserved sites are not exclusively driven by unexpected variations in TF dosage between members of the same species.

We reasoned that variation in YhaJ expression levels between strains would likely result in global binding distinctions and that growth in LB, which normalizes YhaJ dosage, would alleviate these differences. Using ChIP-sequencing (ChIP-seq) of natively expressed YhaJ in each strain's genetic background, we mapped the global binding profile *in vivo* under the two aforementioned conditions, revealing a total of 78

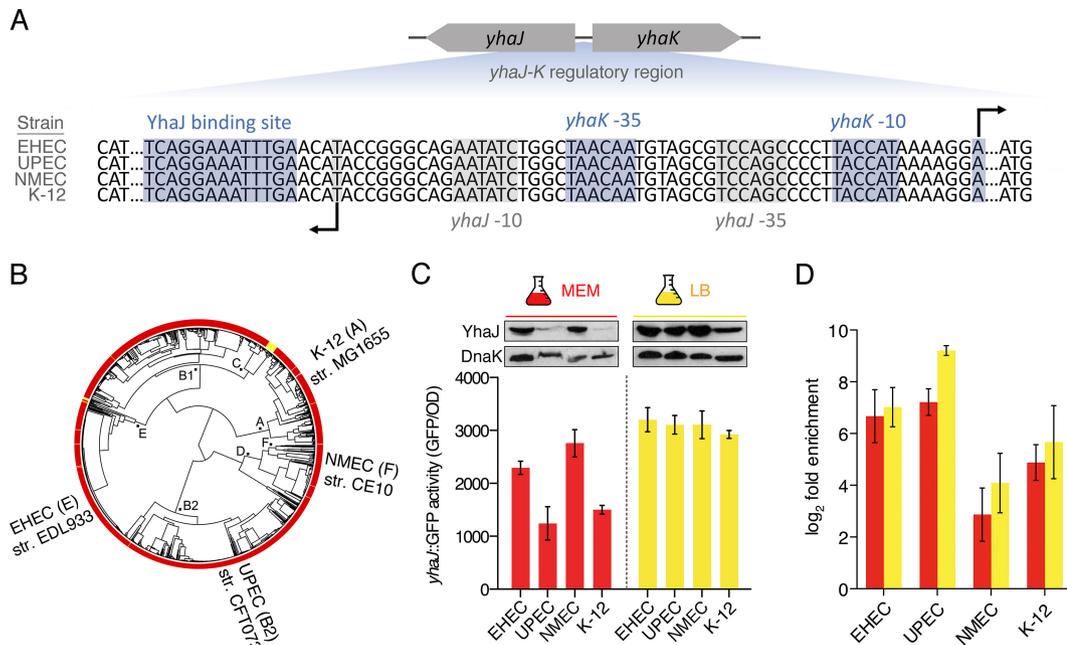


FIG 1 Occupancy dynamics of YhaJ at the *yhaK* regulatory region. (A) Illustration of the *yhaJ*-*yhaK* regulatory region. The expanded aligned DNA sequences depict the conserved $-10/-35$ promoter elements for *yhaJ* (gray) and *yhaK* (blue) as well as the known YhaJ binding site in the four labeled strains. The arrows indicate transcriptional start sites. (B) *E. coli* phylogeny of >1,500 strains. The positions of EHEC, UPEC, NMEC, and *E. coli* K-12 are indicated along with the strains used and the phylogroup they belong to. The red outer layer indicates a conserved YhaJ protein sequence (>80% identity over >80% of the protein-coding sequence), whereas yellow represents a *yhaJ* pseudogene. This figure was generated using the method described in reference 13. str. strain. (C) Analysis of *yhaJ* transcription using a green fluorescent protein (GFP) promoter fusion in MEM (red) or LB (yellow). Corresponding immunoblots showing native YhaJ-FLAG epitope fusion expression are highlighted above. DnaK was used as a loading control. OD, optical density. (D) ChIP-PCR analysis of YhaJ binding enrichment (signal-to-noise ratio) at the *yhaK* regulatory region in MEM (red) and LB (yellow).

significantly enriched peaks ($P \leq 0.01$; two biological replicates) across all strains, including binding sites intragenic in origin (Fig. 2A; see Fig. S2 and Data Set S1 in the supplemental material) (19). Three major observations were made in light of this. First, increased YhaJ expression levels between conditions correlated with an increase in the number of global YhaJ binding sites relative to each strain (EHEC, 23 to 39; UPEC, 7 to 46; NMEC, 12 to 22; K-12, 12 to 34). Second, only ~15% of all binding sites (5/33 in MEM; 12/73 in LB) were occupied in all four strains, regardless of the conditions (Fig. 2B). Third, the majority of strain-specific binding sites identified were not restricted to chromosomal loci unique to each genetic background. While condition-dependent binding sites were not unexpected, these data collectively reveal that the regulatory network of YhaJ is surprisingly heterogenous despite its highly conserved nature across the *E. coli* phylogeny. This suggests that strain-specific regulatory roles for YhaJ are potentially widespread in *E. coli* (5, 14).

Regulatory adaptations in strain-specific loci represent logical repurposing of a TF, particularly for pathogens encoding horizontally acquired virulence factors. We previously demonstrated that this was the case for YhaJ, directly regulating pathogenicity island- and prophage-encoded type 3 secretion system components in EHEC, as well as type 1 fimbriae in UPEC (13, 14). Here, we identified highly significant ($P = 4.9 \times 10^{-52}$) conditional YhaJ binding in the regulatory region of the EHEC type 6 secretion system (T6SS) cluster, exclusively in LB (Fig. 2C) (20). This system plays a role in EHEC virulence and macrophage survival, and this result highlights important flexibility in YhaJ for controlling several virulence factors in a single pathotype (21). Interestingly, UPEC encodes a distinct T6SS, but no YhaJ binding was evident *in vivo*, suggesting pathotype-specific requirements for T6SS regulation (Fig. S3) (20).

While binding to strain-specific loci (particularly virulence-associated loci) is not uncommon for TFs, we were more intrigued by the surprising heterogeneity in global

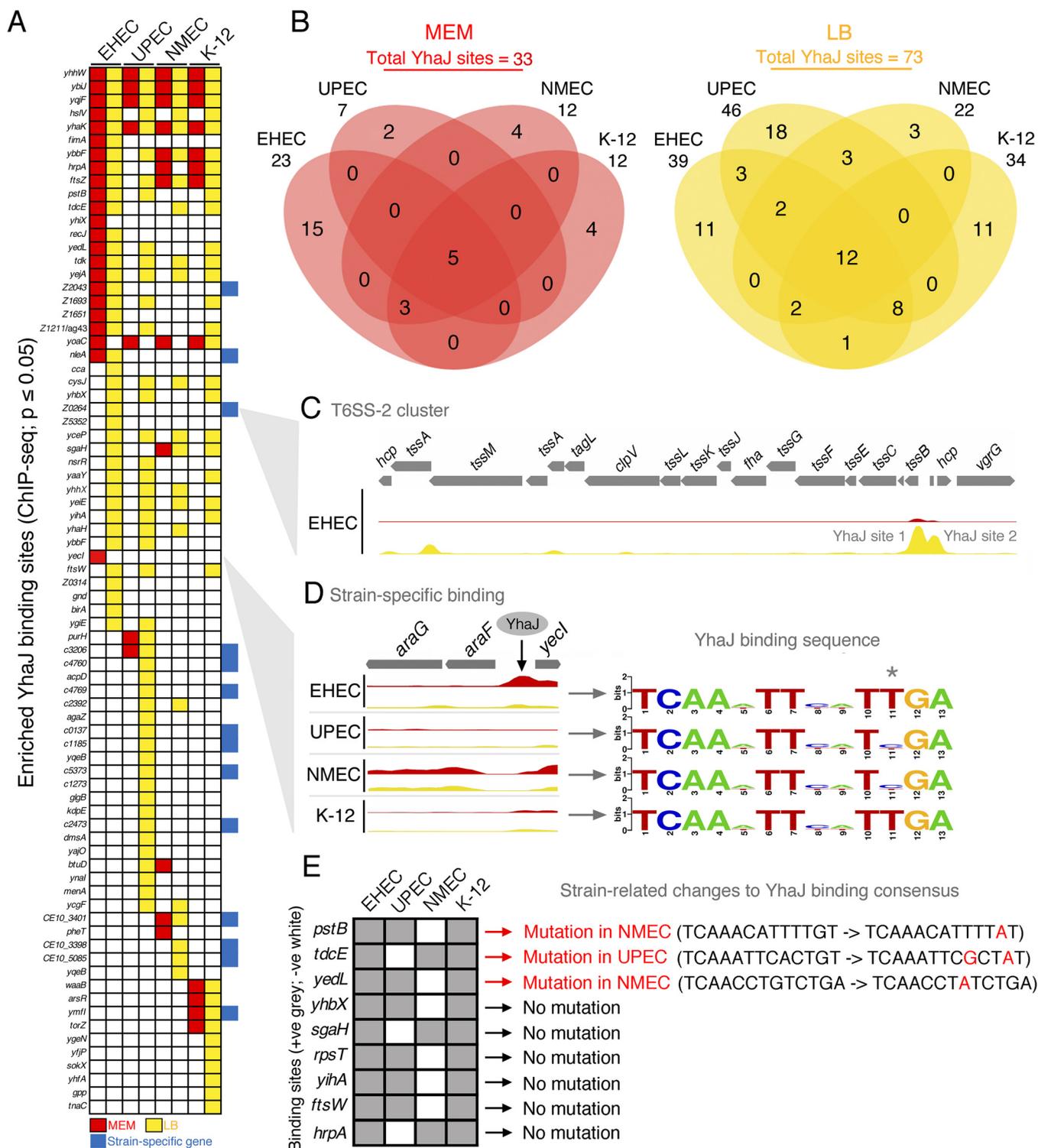


FIG 2 Global chromosomal binding dynamics of YhaJ in evolutionarily distinct *E. coli* isolates. (A) Binding site map indicating significantly enriched YhaJ binding sites ($P < 0.01$; two biological replicates). Red boxes are peaks called in MEM, yellow boxes are peaks called in LB, and blue boxes are strain-specific locations. (B) Venn diagrams highlighting the numbers of binding sites overlapping between and unique to each strain in both conditions. (C) Expanded sequence read track showing YhaJ signal enrichment at the type 6 secretion system (T6SS) regulatory region in EHEC. Red and yellow tracks represent MEM and LB, respectively. (D) Expanded sequence read track showing YhaJ signal enrichment at the *yecI* region for all strains. Sequences that match the YhaJ binding site consensus are indicated on the right. The asterisk highlights a single nucleotide change in UPEC and NMEC. (E) Binding site map of ChIP-seq peaks at the indicated gene regions in all four strains. A gray box indicates YhaJ binding in that strain, whereas a white box represents a lack of significant YhaJ enrichment. Binding sites that match the YhaJ consensus motif are highlighted in red on the right. Specific mutations in binding site sequences associated with a lack of YhaJ enrichment in the particular strain indicated are highlighted by the arrow (black to red sequences). All read tracks were scaled to be comparable to each other for individual gene regions.

Downloaded from <http://mbio.asm.org/> on June 24, 2020 by guest

binding profiles for conserved genes. While YhaJ binding could be driven by growth conditions across all strains (for instance, the known target *yceP*; Fig. S4), we also identified conditional YhaJ binding to conserved gene regions in specific strains. For example, YhaJ bound ($P = 1.35 \times 10^{-7}$) upstream of the EHEC *yeiC* gene (encoding ferritin) exclusively in MEM. LysR-type TFs such as YhaJ recognize partial-dyadic T-N11-A sequences in promoter regions (22). Importantly, analysis of the *yeiC* DNA region revealed that while the YhaJ binding sequence in UPEC and NMEC contained a mutation that affects its partial-dyadic symmetry and possibly functionality, the *E. coli* K-12 motif was identical to the EHEC motif (Fig. 2D). This suggests that strain-specific binding is not exclusively driven by such mutations. We further examined this hypothesis in all cases where binding to a conserved region was absent for one strain. YhaJ motif mutations were present in only three of the nine cases identified (*pstB*, *tdcE*, and *yedL*), revealing that the majority of strain-specific binding distinctions identified are driven by factors independent of mutations to the YhaJ recognition sequence that may include competitive or cooperative binding of other TFs to similar regions in a strain-specific manner (Fig. 2E) (14).

Conclusion. We have observed that a highly conserved TF has adapted its genetic behavior drastically on an individual level to create strain-specific chromosomal interactions in *E. coli*. These distinctions are amplified according to TF dosage and are not driven purely by binding site mutations or attraction to strain-specific genetic loci. The resulting binding profiles represent a previously underappreciated diversity in intraspecies regulatory potential and highlight that global gene regulation studies should not rely on single model strains. Given the ecological diversity of *E. coli* as a species and the fact that it dedicates a large proportion of its genome to regulation (~6% in *E. coli* K-12 [6]), we anticipate that this is a widespread phenomenon allowing the emergence of strain-specific regulatory networks.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

TEXT S1, DOCX file, 0.02 MB.

FIG S1, TIF file, 0.3 MB.

FIG S2, TIF file, 0.4 MB.

FIG S3, TIF file, 0.2 MB.

FIG S4, TIF file, 0.2 MB.

TABLE S1, DOCX file, 0.01 MB.

TABLE S2, DOCX file, 0.01 MB.

TABLE S3, DOCX file, 0.01 MB.

DATA SET S1, XLSX file, 0.04 MB.

ACKNOWLEDGMENTS

J.P.R.C. is supported by a fellowship from the Faculty of Medical Sciences (Newcastle University) and a Springboard award from the Academy of Medical Sciences [SBF005\1029]. N.O. is supported by a Tenovus Scotland small pilot grant. A.J.R. acknowledges support from the Biotechnology and Biological Sciences Research Council [BB/M029646/1, BB/R006539/1].

REFERENCES

- Browning DF, Busby SJW. 2016. Local and global regulation of transcription initiation in bacteria. *Nat Rev Microbiol* 14:638–650. <https://doi.org/10.1038/nrmicro.2016.103>.
- Seshasayee ASN, Sivaraman K, Luscombe NM. 2011. An overview of prokaryotic transcription factors, p 7–24. *In* Hughes TR (ed), *A handbook of transcription factors*. Subcellular biochemistry, vol 52. Springer, Berlin, Germany.
- Connolly JPR, Finlay BB, Roe AJ. 2015. From ingestion to colonization: the influence of the host environment on regulation of the LEE encoded type III secretion system in enterohaemorrhagic *Escherichia coli*. *Front Microbiol* 6:568. <https://doi.org/10.3389/fmicb.2015.00568>.
- Turner NCA, Connolly JPR, Roe AJ. 2018. Control freaks — signals and cues governing the regulation of virulence in attaching and effacing pathogens. *Biochem Soc Trans* 47:229–238. <https://doi.org/10.1042/BST20180546>.
- O’Boyle N, Turner NCA, Roe AJ, Connolly JPR. 2020. Plastic circuits: regulatory flexibility in fine tuning pathogen success. *Trends Microbiol* 28:360–371. <https://doi.org/10.1016/j.tim.2020.01.002>.
- Perez JC, Groisman EA. 2009. Evolution of transcriptional regulatory circuits in bacteria. *Cell* 138:233–244. <https://doi.org/10.1016/j.cell.2009.07.002>.
- Blattner FR, Plunkett G, Bloch CA, Perna NT, Burland V, Riley M, Collado-

- Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* 277:1453–1462. <https://doi.org/10.1126/science.277.5331.1453>.
8. Perna NT, Plunkett G, Burland V, Mau B, Glasner JD, Rose DJ, Mayhew GF, Evans PS, Gregor J, Kirkpatrick HA, Pósfai G, Hackett J, Klink S, Boutin A, Shao Y, Miller L, Grotbeck EJ, Davis NW, Lim A, Dimalanta ET, Potamousis KD, Apodaca J, Anantharaman TS, Lin J, Yen G, Schwartz DC, Welch RA, Blattner FR. 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature* 409:529–533. <https://doi.org/10.1038/35054089>.
 9. Welch RA, Burland V, Plunkett G, Redford P, Roesch P, Rasko D, Buckles EL, Liou S-R, Boutin A, Hackett J, Stroud D, Mayhew GF, Rose DJ, Zhou S, Schwartz DC, Perna NT, Mobley HLT, Donnenberg MS, Blattner FR. 2002. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *Proc Natl Acad Sci U S A* 99:17020–17024. <https://doi.org/10.1073/pnas.252529799>.
 10. Lu S, Zhang X, Zhu Y, Kim KS, Yang J, Jin Q. 2011. Complete genome sequence of the neonatal-meningitis-associated *Escherichia coli* strain CE10. *J Bacteriol* 193:7005. <https://doi.org/10.1128/JB.06284-11>.
 11. Brzuszkiewicz E, Brüggemann H, Liesegang H, Emmerth M, Olschläger T, Nagy G, Albermann K, Wagner C, Buchrieser C, Emody L, Gottschalk G, Hacker J, Dobrindt U. 2006. How to become a uropathogen: comparative genomic analysis of extraintestinal pathogenic *Escherichia coli* strains. *Proc Natl Acad Sci U S A* 103:12879–12884. <https://doi.org/10.1073/pnas.0603038103>.
 12. Kaper JB, Nataro JP, Mobley HL. 2004. Pathogenic *Escherichia coli*. *Nat Rev Microbiol* 2:123–140. <https://doi.org/10.1038/nrmicro818>.
 13. Connolly JPR, Gabrielsen M, Goldstone RJ, Grinter R, Wang D, Cogdell RJ, Walker D, Smith DGE, Roe AJ. 2016. A highly conserved bacterial D-serine uptake system links host metabolism and virulence. *PLoS Pathog* 12:e1005359. <https://doi.org/10.1371/journal.ppat.1005359>.
 14. Connolly JPR, O'Boyle N, Turner NCA, Browning DF, Roe AJ. 2019. Distinct intraspecies virulence mechanisms regulated by a conserved transcription factor. *Proc Natl Acad Sci U S A* 116:19695–19704. <https://doi.org/10.1073/pnas.1903461116>.
 15. King T, Ishihama A, Kori A, Ferenci T. 2004. A regulatory trade-off as a source of strain variation in the species *Escherichia coli*. *J Bacteriol* 186:5614–5620. <https://doi.org/10.1128/JB.186.17.5614-5620.2004>.
 16. Spira B, Hu X, Ferenci T. 2008. Strain variation in ppGpp concentration and RpoS levels in laboratory strains of *Escherichia coli* K-12. *Microbiology* 154:2887–2895. <https://doi.org/10.1099/mic.0.2008/018457-0>.
 17. Ferenci T, Galbiati HF, Betteridge T, Phan K, Spira B. 2011. The constancy of global regulation across a species: the concentrations of ppGpp and RpoS are strain-specific in *Escherichia coli*. *BMC Microbiol* 11:62. <https://doi.org/10.1186/1471-2180-11-62>.
 18. Palevsky N, Shemer B, Connolly JPR, Belkin S. 2016. The highly conserved *Escherichia coli* transcription factor yhaJ regulates aromatic compound degradation. *Front Microbiol* 7:1490. <https://doi.org/10.3389/fmicb.2016.01490>.
 19. Shimada T, Ishihama A, Busby SJW, Grainger DC. 2008. The *Escherichia coli* RutR transcription factor binds at targets within genes as well as intergenic regions. *Nucleic Acids Res* 36:3950–3955. <https://doi.org/10.1093/nar/gkn339>.
 20. Journet L, Cascales E. 1 February 2016, posting date. The type VI secretion system in *Escherichia coli* and related species. *EcoSal Plus* 2016 <https://doi.org/10.1128/ecosalplus.ESP-0009-2015>.
 21. Wan B, Zhang Q, Ni J, Li S, Wen D, Li J, Xiao H, He P, Ou HY, Tao J, Teng Q, Lu J, Wu W, Yao YF. 2017. Type VI secretion system contributes to enterohemorrhagic *Escherichia coli* virulence by secreting catalase against host reactive oxygen species (ROS). *PLoS Pathog* 13:e1006246. <https://doi.org/10.1371/journal.ppat.1006246>.
 22. Maddocks SE, Oyston PCF. 2008. Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins. *Microbiology* 154:3609–3623. <https://doi.org/10.1099/mic.0.2008/022772-0>.