

# World Journal of *Clinical Infectious Diseases*

*World J Clin Infect Dis* 2020 May 15; 10(1): 1-23





**MINIREVIEWS**

- 1 COVID-19 compared to other epidemic coronavirus diseases and the flu  
*Ayukekbong JA, Ntemgwa ML, Ayukekbong SA, Ashu EE, Agbor TA*

**ORIGINAL ARTICLE**

**Basic Study**

- 14 GadE regulates *fliC* gene transcription and motility in *Escherichia coli*  
*Schwan WR, Flohr NL, Multerer AR, Starkey JC*

**ABOUT COVER**

Editor-in-Chief of *World Journal of Clinical Infectious Diseases*, Joao Mesquita, DVM, MSc, PhD, Professor, Epidemiology Research Unit, Polytechnic Institute of Viseu; Public Health Institute of the University of Porto, Porto 3500-606, Portugal

**AIMS AND SCOPE**

The primary aim of *World Journal of Clinical Infectious Diseases* (WJCID, *World J Clin Infect Dis*) is to provide scholars and readers from various fields of infectious diseases with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCID mainly publishes articles reporting research results and findings obtained in the field of infectious diseases and covering a wide range of topics including community-acquired infections, cross infection, eye infections, focal infection, infectious gingivitis, intraabdominal infections, laboratory infection, Ludwig's angina, necrotizing ulcerative periodontitis, opportunistic infections, pelvic infection, pregnancy complications, prosthesis-related infections, reproductive tract infections, respiratory tract infections, sepsis, sexually transmitted diseases, skin diseases, soft tissue infections, suppuration, toxemia, urinary tract infections, and wound infection.

**INDEXING/ABSTRACTING**

*World Journal of Clinical Infectious Diseases* is now indexed in China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (CSTJ), and Superstar Journals Database.

**RESPONSIBLE EDITORS FOR THIS ISSUE**

Responsible Electronic Editor: *Ji-Hong Liu*

Proofing Production Department Director: *Yun-Xiaojuan Wu*

Responsible Editorial Office Director: *Ya-Juan Ma*

**NAME OF JOURNAL**

*World Journal of Clinical Infectious Diseases*

**ISSN**

ISSN 2220-3176 (online)

**LAUNCH DATE**

December 30, 2011

**FREQUENCY**

Irregular

**EDITORS-IN-CHIEF**

Joao Mesquita, Caterina Sagnelli, Wei Wang

**EDITORIAL BOARD MEMBERS**

<https://www.wjgnet.com/2220-3176/editorialboard.htm>

**PUBLICATION DATE**

May 15, 2020

**COPYRIGHT**

© 2020 Baishideng Publishing Group Inc

**INSTRUCTIONS TO AUTHORS**

<https://www.wjgnet.com/bpg/gerinfo/204>

**GUIDELINES FOR ETHICS DOCUMENTS**

<https://www.wjgnet.com/bpg/GerInfo/287>

**GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH**

<https://www.wjgnet.com/bpg/gerinfo/240>

**PUBLICATION ETHICS**

<https://www.wjgnet.com/bpg/GerInfo/288>

**PUBLICATION MISCONDUCT**

<https://www.wjgnet.com/bpg/gerinfo/208>

**ARTICLE PROCESSING CHARGE**

<https://www.wjgnet.com/bpg/gerinfo/242>

**STEPS FOR SUBMITTING MANUSCRIPTS**

<https://www.wjgnet.com/bpg/GerInfo/239>

**ONLINE SUBMISSION**

<https://www.f6publishing.com>

## Basic Study

GadE regulates *fliC* gene transcription and motility in *Escherichia coli*

William R Schwan, Nicole L Flohr, Abigail R Multerer, Jordan C Starkey

**ORCID number:** William R Schwan (0000-0003-3076-1815); Nicole L Flohr (0000-0003-0445-124X); Abigail R Multerer (0000-0001-6789-6268); Jordan C Starkey (0000-0001-9160-8588).

**Author contributions:** Schwan WR, Flohr NL, Multerer AR, and Starkey JC designed the research, performed the research; Schwan WR, Flohr NL, and Multerer AR analyzed the data; Schwan WR wrote the paper.

**Supported by** the National Institutes of Health, No. 1R15AI-065432.

**Institutional review board**

**statement:** No humans or samples from human were used in this study.

**Institutional animal care and use**

**committee statement:** No animals were used in this study.

**Conflict-of-interest statement:** The authors report no conflict of interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build

William R Schwan, Nicole L Flohr, Abigail R Multerer, Jordan C Starkey, Department of Microbiology, University of Wisconsin-La Crosse, La Crosse, WI 54601, United States

**Corresponding author:** William R Schwan, PhD, Professor, Department of Microbiology, University of Wisconsin-La Crosse, 1725 State St, La Crosse, WI 54601, United States. [wschwan@uwlax.edu](mailto:wschwan@uwlax.edu)

## Abstract

## BACKGROUND

*Escherichia coli* (*E. coli*) express flagella to ascend human urinary tracts. To survive in the acidic pH of human urine, *E. coli* uses the glutamate decarboxylase acid response system, which is regulated by the GadE protein.

## AIM

To determine if growth in an acidic pH environment affected *fliC* transcription and whether GadE regulated that transcription.

## METHODS

A *fliC-lacZ* reporter fusion was created on a single copy number plasmid to assess the effects of acidic pH on *fliC* transcription. Further, a  $\Delta$ *gadE* mutant strain of a uropathogenic *E. coli* was created and tested for motility compared to the wild-type strain.

## RESULTS

*Escherichia coli* cells carrying the *fliC-lacZ* fusion displayed significantly less *fliC* transcription when grown in an acidic pH medium compared to when grown in a neutral pH medium. Transcription of *fliC* fell further when the *E. coli* was grown in an acidic pH/high osmolarity environment. Since GadE is a critical regulator of one acid response system, *fliC* transcription was tested in a *gadE* mutant strain grown under acidic conditions. Expression of *fliC* was derepressed in the *E. coli gadE* mutant strain grown under acidic conditions compared to that in wild-type bacteria under the same conditions. Furthermore, a *gadE* mutation in a uropathogenic *E. coli* background exhibited significantly greater motility than the wild-type strain following growth in an acidic medium.

## CONCLUSION

Together, our results suggest that GadE may down-regulate *fliC* transcription and motility in *E. coli* grown under acidic conditions.

upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited Manuscript

**Received:** February 7, 2020

**Peer-review started:** February 7, 2020

**First decision:** March 5, 2020

**Revised:** March 25, 2020

**Accepted:** May 5, 2020

**Article in press:** May 5, 2020

**Published online:** May 15, 2020

**P-Reviewer:** García-Elorriaga G, Nagata T, Song G

**S-Editor:** Wang J

**L-Editor:** A

**E-Editor:** Liu JH



**Key words:** *Escherichia coli*; Flagella; GadE; Motility; Acid response; *fliC*

©The Author(s) 2020. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** *Escherichia coli* (*E. coli*) is the number one cause of urinary tract infections in women. The infections are the result of the *E. coli* cells ascending the urinary tract via flagella presented on the outside of the cells. In this study, we have shown that *E. coli* grown in a low pH/high-osmolarity environment display transcriptional repression of the *fliC* flagellin subunit gene. Furthermore, we demonstrate that GadE may regulate *fliC* transcription and subsequent motility of the *E. coli* cells.

**Citation:** Schwan WR, Flohr NL, Multerer AR, Starkey JC. GadE regulates *fliC* gene transcription and motility in *Escherichia coli*. *World J Clin Infect Dis* 2020; 10(1): 14-23

**URL:** <https://www.wjgnet.com/2220-3176/full/v10/i1/14.htm>

**DOI:** <https://dx.doi.org/10.5495/wjcid.v10.i1.14>

## INTRODUCTION

In the United States, approximately 10.5 million women suffer from a urinary tract infection each year. Around 80% of urinary tract infection are caused by uropathogenic *Escherichia coli* (UPEC), resulting in over 100000 hospitalizations and an approximate cost of \$ 3.5 billion per year<sup>[1-3]</sup>. UPEC sometimes ascend all of the way to the kidneys, causing life-threatening pyelonephritis in some of the women<sup>[2,3]</sup>. The ability of *Escherichia coli* (*E. coli*) to move up the human urinary tract is due to the presence of flagella expressed by the bacteria<sup>[4-7]</sup>.

Bacterial flagella allow the directional movement of *E. coli* based upon a chemotactic response<sup>[8,9]</sup>. Several genes are involved in the expression of flagella, although *fliC* encodes the flagellin subunits that comprise the bulk of a flagellum structure<sup>[10]</sup>. Several studies have shown the importance of flagella in UPEC pathogenesis<sup>[4-7,11]</sup>. For instance, several studies have examined the prevalence of the *fliC* gene in UPEC strains. One study showed the prevalence of the *fliC* gene in UPEC strains varied from 84% (community-acquired) to 95% (nosocomial-acquired)<sup>[12]</sup>, whereas another study reported that only 16% of the UPEC strains had the *fliC* gene<sup>[13]</sup>. Part of the disparity in the frequency of *fliC* gene prevalence could be due to the respective primers used in each study. Certainly, UPEC flagella are critical for ascension out of the bladder into the kidneys of an animal host. Within a mouse or human urinary tract, UPEC are continuously bathed in urine. Typically, human and murine urine will have a slightly acidic pH and variations in osmolality<sup>[14-16]</sup>, although the osmolality within murine urine is usually higher than human urine<sup>[15]</sup>. Hence, pH is one critical environmental factor found in the urinary tract.

Within *E. coli*, homeostasis in an acidic environment is mediated by at least five acid response (AR) systems<sup>[17-21]</sup>. System two (AR2) is induced in stationary phase and requires a glutamate decarboxylase and a glutamate: γ-aminobutyric acid antiporter. AR2 is the predominant and best characterized of the five AR system pathways<sup>[22-25]</sup>. The AR2 requires the antiporter GadC and two inducible glutamate decarboxylases: GadA and GadB. The antiporter is responsible for transporting glutamate into the cell while transporting the product of glutamate decarboxylation, glutamate: γ-aminobutyric acid, out of the cell<sup>[22,24-30]</sup>. GadE, belonging to the LuxR family of regulatory proteins<sup>[31]</sup>, has been identified as the central transcriptional activator of *gadA/BC*, and provides the primary means of *gadA/BC* activation<sup>[32,33]</sup>. Microarray studies done under acidic conditions originally identified the *yhiE* gene (renamed *gadE*), which was found to encode for this transcriptional regulator protein<sup>[31]</sup>. GadE binds to a 20-bp sequence (GAD box: 5'-TTAGGATTTTGTATTATAA-3') located -63 bp from the transcriptional start site of both *gadA* and the *gadBC* operon and is necessary for expression of these genes under all conditions<sup>[28,34,35]</sup>.

In this study, we have studied the role GadE may play in *E. coli* flagella expression. Through the use of a *gadE* mutant, a *fliC-lacZ* reporter system, and a motility assay; we demonstrate that GadE regulates transcription of *fliC* in *E. coli*, which in turn affects bacterial motility.

## MATERIALS AND METHODS

### Bacterial strains, plasmids, and media

All of the bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* strain NU149 is a clinical isolate obtained from a patient with cystitis<sup>[36]</sup>. The *E. coli* strain DH5 $\alpha$  was used to construct the *fliC-lacZ* reporter system. *E. coli* strains MC4100 (supplied by Linda Kenney) and EK227 (supplied by John Foster) were subsequently tested under various pH and osmotic conditions with the *fliC-lacZ* reporter system. The  $\Delta$ *gadE* strain EF1007 and  $\Delta$ *gadE*/pPCRScrip Amp *gadE* strain EF1083 were also supplied by John Foster. Multicopy plasmid pUJ9<sup>[37]</sup> and single copy plasmid pPP2-6<sup>[38]</sup> were used for cloning. The pUJ9 plasmid contains a promoterless *lacZ* gene and an ampicillin antibiotic resistance gene. Plasmid pPP2-6 is a single copy plasmid with a multiple cloning site that possesses a chloramphenicol resistance gene<sup>[38]</sup>. The pPCRScrip Amp *gadE* plasmid had the *gadE* gene cloned into the multicopy plasmid pPCRScrip Amp<sup>[33]</sup>. Luria Agar (LA) supplemented with 12.5  $\mu$ g/mL chloramphenicol was used to grow the recombinant *E. coli* cells containing the reporter system. Luria Bertani (LB) broth containing 1% glycerol at pH's ranging from 5.5 to 8.0 was used to test pH ranges, and LB broth (pH 5.5 and pH 7.0, 1% glycerol, 0.1 mol/L Na<sub>3</sub>PO<sub>4</sub> buffering) coupled with osmotic variation of 0 to 400 mmol/L NaCl was used to gauge pH plus osmotic changes<sup>[38]</sup>. Under these growth conditions, the recombinant *E. coli* strains were assayed for  $\beta$ -galactosidase activity.

### Construction of the *fliC-lacZ* fusion

Oligonucleotide primers FliC1 (5'-GAGAGAATTTCGATGAAATACTTGCCATGC-3') and FliC2 (5'-AGAAGGATCCAGACGCTGGATAGAACTC-3') specific for a 397-bp segment of the *E. coli* strain NU149 *fliC* promoter were amplified with the *Bam*HI and *Eco*RI restriction endonuclease sites flanking the DNA promoter sequence. Polymerase chain reaction (PCR) amplification using these primers was set up as follows: An initial denaturation of five minutes, then 35 cycles 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, finishing with a 7 min elongation at 72 °C after the 35th cycle. Chromosomal DNA from *E. coli* strain NU149 extracted with a PurElute Bacterial Genomic kit (Edge Biosystems, Mountain View, CA, United States) served as the template in the PCR. The 397-bp product was visualized on a 0.8% agarose gel containing ethidium bromide with a 1 kb ladder (New England Biolabs, Ipswich, MA, United States) served as the molecular weight standard.

The PCR amplified 397-bp *fliC* promoter DNA fragment was passed through a Microcon 30 filter (MilliporeSigma, Burlington, VT, United States) to concentrate the DNA. Subsequently, the DNA was digested with the restriction endonucleases *Eco*RI and *Bam*HI (New England Biolabs). The digested DNA fragment was ligated to *Eco*RI/*Bam*HI digested pUJ9 plasmid DNA and transformed into competent DH5 $\alpha$  cells. The resulting transformants were selected on LA containing 100  $\mu$ g/mL ampicillin and X-Gal (Promega, Madison, WI, United States). Blue colonies were screened for  $\beta$ -galactosidase<sup>[39]</sup> and the plasmid DNA was extracted with a QIAprep kit (Qiagen, Valencia, CA, United States) to verify the appropriate size. One recombinant plasmid, pNK1-1, was carried further in the process. This plasmid DNA was digested with the restriction endonuclease *Not*I (New England Biolabs) and ligated to *Not*I cut pPP2-6 DNA. Following ligation, the DNA was transformed into DH5 $\alpha$  and clones were selected on LA containing 12.5  $\mu$ g/mL chloramphenicol and X-Gal. One clone, pNK2-29, was selected for *in vitro* analysis.

### Galactosidase assays

Galactosidase assays were performed on DH5 $\alpha$ /pNK2-29 and MC4100/pPP2-6 cells grown in LB media at various pH and in the presence and absence of NaCl at pH 5.5 and 7.0<sup>[39]</sup>. Bacteria were grown mid-logarithmically and  $\beta$ -galactosidase activity on the sodium dodecyl sulfate and CHCl<sub>3</sub> permeabilized cells. The mean values + standard deviation was calculated from at least three separate experiments for each bacterial strain.

### Creation of a $\Delta$ *gadE* mutation in uropathogenic *E. coli* strain NU149

To create a deletion mutation of the *gadE* gene, the red recombinase system described by Datsenko and Wanner<sup>[40]</sup> was used. Briefly, the primer pair GadE1 (5'-GATGACATATTCGAAACGATAACGGCTAAGGAGCAAGTTTGTGTAGGCTGGAGCTGCTTCG-3') and GadE2 (5'-TCGTCATGCCAGCCATGAATTTCA-GTTGCTTATGTCCTGACATATGAATATCCTCCTTAG-3') was used to create a PCR product, using pKD4 plasmid DNA as a template. The PCR conditions that were used were an initial denaturation at 95 °C for 5 min followed by 35 cycles of 95 °C, 1 min; 57 °C, 1 min; and 72 °C, 2 min. The resulting PCR product was concentrated and separated on a 0.8% agarose gel, cut out, and the DNA extracted from the agarose gel.

**Table 1 Bacterial strains and plasmids used in the study**

Strain/plasmid	Description	Source
Strain		
DH5a MCR	Transformation efficient strain	Gibco/BRL
MC4100	<i>E. coli</i> K-12 strain	Linda Kenney
EK227	<i>E. coli</i> K-12 strain	[53]
EF1007	<i>gadE</i> ::Km in EK227	[54]
EF1083	<i>gadE</i> ::Km/pPCRScrip Amp <i>gadE</i>	[33]
NU149	Clinical isolate	[36]
NU149 <i>gadE</i>	$\Delta$ <i>gadE</i> mutation in NU149	This study
NU149 LacZ1	$\Delta$ <i>lacZ</i> mutation in NU149	This study
Plasmid		
pUJ9	Promoterless <i>lacZ</i> gene, Ap <sup>R</sup>	[37]
pPP2-6	Single copy plasmid, Cm <sup>R</sup>	[38]
pKD4	Flp recombinase sites, Km <sup>R</sup>	[40]
pKD46	Red recombinase, Ap <sup>R</sup>	[40]
pCP20	Flp recombinase, Ap <sup>R</sup>	[40]
pNK2-29	<i>fliC</i> :: <i>lacZ</i> on pPP2-6, Ap <sup>R</sup>	This study
pPCRScrip Amp <i>gadE</i>	<i>gadE</i> on pPCRScrip Amp	[33]

With this purified PCR product, an electroporation was performed on strain NU149/pKD46 cells as described previously<sup>[40]</sup>, selecting for transformants on LA with 40 µg/mL kanamycin. One transformant, NU149 *gadE*, was chosen for further analysis. To remove the kanamycin resistance gene, plasmid pCP20 was introduced into NU149 *gadE* by electroporation. The resulting strain was processed as noted previously<sup>[6]</sup>. To confirm the *gadE* deletion, a PCR-based assay was used with the GadE5 (5'-ACAGGGCTTTTGGCAGTTGAA-3') and GadE6 (5'-AAATATTAGCGTCGACGTGA-3') primers. The PCR conditions that were used were an initial denaturation at 95 °C for 5 min followed by 30 cycles of 95 °C, 1 min; 57 °C, 1 min; and 72 °C, 2 min. This  $\Delta$ *gadE* mutation was complemented by electroporating the pPCRScrip Amp *gadE* plasmid into NU149 *gadE* and selecting for transformants on LA with 100 µg/mL ampicillin. The wild-type NU149 strain was used a positive control and *Staphylococcus aureus* genomic DNA was used as a negative control.

### Construction of $\Delta$ *lacZ* mutation in uropathogenic *E. coli* strain NU149

To construct the  $\Delta$ *lacZ* mutation in UPEC strain NU149, the procedure described above was used. The LacZ1 (5'-CCTTACGCGAAATACGGGCAGACATGGCCTGCCCCGGTTAT

TACATATGAATATCCTCCTTAG-3') and LacZ2 (5'-TGGAATTGTGAGCGG-ATAACAA

TTTCACACAGGAAACAGCTTGTGTAGGCTGGAGCTGCTTCG-3') primer pair were used to create the PCR product using the amplification conditions noted above. To confirm the  $\Delta$ *lacZ* mutation, the LacZ3 (5'-ATGAAACGCCGAGTTAACGC-3') and LacZ4 (5'-AGCTGGCGTAATAGCGAAGA-3') primers were used in the PCR amplification conditions described above. Plasmid pNK2-29 was electroporated into strain NU149 and colonies were selected on MacConkey containing 12.5 mg/mL chloramphenicol.

### Soft agar assay for motility

A soft agar motility test was performed as previously described<sup>[41]</sup> for the wild-type *vs* *gadE* mutant and complemented mutant analysis. Each strain was inoculated into the center of the agar plate and the amount of bacterial spread measured after 24 h post-inoculation. The motility assays were repeated two more times on separate days.

### Statistical analyses

A two-tailed Student's *t*-test was used to calculate statistical variation with a *P* < 0.05 considered significant.

## RESULTS

### Examination of the *fliC::lacZ* fusion at different pHs

To assess whether pH affected the transcription of our *fliC-lacZ* fusion plasmid, the pH of buffered LB medium was adjusted to 5.5 to 8.0 by using 0.1 M Na<sub>3</sub>PO<sub>4</sub> buffering and glycerol to maintain the pH<sup>[38]</sup>. The resulting media were inoculated with MC4100/pNK2-29 and the  $\beta$ -galactosidase activities of mid-logarithmic-phase cells were determined. The optimal pH for *fliC* expression was found to be at pH 7.0 (1111 Miller units; Table 2). As the pH shifted to the acidic range, *fliC* transcription declined until there was a significant 3.9-fold difference observed comparing *fliC* transcription at pH 7.0 compared to pH 5.5 (288 Miller units,  $P < 0.01$ ). When the pH of the buffered LB was raised into the alkaline range, there was a slight decline in *fliC* transcription that was 1.5-fold lower at pH 8.0 (738 Miller units,  $P < 0.05$ ) *vs* growth in pH 7.0 medium. These results indicate that pH alone affects *fliC* transcription.

### Effects of pH and osmotic conditions together on *fliC::lacZ* transcription

In an environment such as the human or murine urinary tract, fluctuations in both pH and osmolarity can occur<sup>[14-16]</sup>. To determine if the combination of acidic pH and high osmolarity affect *fliC* transcription, MC4100/pNK2-29 was grown in buffered pH with variation in both the pH (5.5 and 7.0) and the osmolarity (0 to 400 mmol/L NaCl). When MC4100/pNK2-29 was grown in pH 7.0/low-osmolarity (0 mmol/L NaCl) LB, *fliC* transcription was the highest (1,132 Miller units, Table 3). An increase in the osmolarity to 400 mmol/L NaCl in the pH 7.0 LB caused *fliC* transcription to significantly fall by 2.5-fold (454 Miller units,  $P < 0.01$ ) compared to growth in the pH 7.0 low-osmolarity LB. *E. coli* with the pNK2-29 plasmid grown in pH 5.5/low-osmolarity conditions displayed *fliC* transcription of 308 Miller units (Table 3); however, *fliC* transcription dropped almost 5-fold to 62 Miller Units ( $P < 0.01$ ) as the osmolarity increased to 400 mmol/L NaCl. A comparison of *fliC* transcription in *E. coli* grown in pH 7.0/low-osmolarity LB to the *E. coli* population grown in pH 5.5/high-osmolarity LB showed a highly significant 18.2-fold change ( $P < 0.001$ ). Thus, a growth environment possessing both an acidic pH and high osmolarity substantially repressed *fliC* transcription in the *E. coli* K-12 strain.

To determine if the same *fliC* transcriptional changes occurred in a UPEC strain, a  $\Delta$ *lacZ* mutation was created in UPEC strain NU149. The pNK2-29 plasmid containing the *fliC-lacZ* fusion was moved into *E. coli* strain NU149 LacZ1 and the same environmental conditions tested for the *E. coli* K-12 strain were used. Growth of NU149 LacZ1/pNK2-29 in pH 7.0 with no added NaCl displayed the highest *fliC* transcription (1353 Miller Units, Table 3), whereas *fliC* transcription significantly fell 3.06-fold when the strain was grown in pH 5.5 LB (442 Miller Units,  $P < 0.01$ ). An increase in the osmolarity to 400 mM NaCl in pH 7.0 LB caused *fliC* transcription to fall 2.77-fold (489 Miller Units,  $P < 0.01$ ). Moreover, the growth of NU149 LacZ1/pNK2-29 in pH 5.5 LB with 400 mM added NaCl showed the lowest level of *fliC* transcription (147 Miller Units) that was 9.2-fold lower than when grown in pH 7.0 no added salt medium ( $P < 0.01$ ). Overall, the *fliC* transcription results in the UPEC strain mirrored the *E. coli* K-12 strain's results.

### Transcription of *fliC* was affected by the *gadE* mutation in *E. coli* grown in acidic pH media

As shown above, acidic pH growth conditions led to lower *fliC* transcription compared to transcription in neutral pH growth conditions. Previous work has shown that the glutamate decarboxylase system is critical for acid resistance in *E. coli* and GadE is an important regulator of this AR system<sup>[31-33]</sup>. We then asked whether GadE might also regulate *fliC* transcription under acidic growth conditions. We examined an *E. coli* K-12 wild-type strain, a *gadE* mutant strain as well as a complemented *gadE* mutant strain all of which contained the *fliC-lacZ* pNK2-29 plasmid. The strains were grown in buffered LB set at pH 5.5 or 7.0 with (400 mmol/L) or without (0 mmol/L) added NaCl and monitored for galactosidase activity. Derepression of *fliC* transcription occurred in the *gadE* mutant grown in acidic pH LB (Table 4). After growth in pH 5.5/low-osmolarity medium, the *gadE* mutant strain (1742 Miller units) exhibited a 3.2-fold increase in *fliC* transcription, compared to the wild-type strain (540 Miller units,  $P < 0.001$ ), which indicated that GadE repressed *fliC* under acidic conditions. Complementation with an intact *gadE* gene reduced the activity below the wild-type levels to 295 Miller units, below even wild-type levels, confirming the repressive effect of GadE on *fliC* expression. The repressive effect of GadE on *fliC* expression was reduced in pH 7.0/low-osmolarity medium with the *gadE* mutant strain showing only slightly higher *fliC* transcription (2196 Miller units) *vs* the *gadE*+ wild-type strain (1520 Miller units,  $P < 0.01$ ). However, when the growth conditions were changed to a high osmolarity environment (400 mmol/L NaCl), the *gadE*

**Table 2** Effect of pH on *fliC::lacZ* gene transcription in *Escherichia coli* strain MC4100/pNK2-29 grown in buffered Luria Bertani media

pH	Gal activity <sup>1</sup>
5.5	288 ± 81.5
6	528 ± 82.5
6.5	629 ± 114
7	1111 ± 110
7.5	932 ± 190
8	738 ± 125

<sup>1</sup>Galactosidase activity measured as Miller units.

mutation had no significant effect on *fliC* transcription (540 Miller units). A change to a pH 5.5/ high-osmolarity environment caused a further repression of *fliC* transcription (165 Miller units,  $P < 0.05$ ) that was significant.

### A *gadE* mutation affects uropathogenic *E. coli* motility

The data above suggested that GadE may repress *fliC* transcription when *E. coli* is grown under acidic pH conditions. Since transcriptional differences do not always translate into protein level differences or functional differences, the effects of a *gadE* mutation on *E. coli* motility was next tested. First, motility was tested using the *E. coli* K-12 strain EF227 (wild-type), EK1007 (*gadE* mutation), and EF1083 (*gadE* mutation complemented with the pPCRScrip Amp *gadE* plasmid). All strains were grown in pH 5.5 buffered LB and spotted onto motility agar plates. Wild-type *E. coli* strain EF227 displayed an 8.33 mm spread diameter, whereas strain EF1007 showed a significantly larger spread diameter of 45 mm ( $P < 0.001$ , Table 5). When the *gadE* mutation was complemented in strain 1083, the spread diameter dropped below the wild-type level (6.67 mm diameter).

A *gadE* mutation was also created in the uropathogenic *E. coli* clinical isolate NU149 using a  $\lambda$ red recombinase system. The NU149, NU149 *gadE*, and NU149 *gadE*/pPCRScrip Amp *gadE* strains were grown in pH 5.5 buffered LB and spotted onto motility agar plates. Wild-type *E. coli* strain NU149 had a 10.67 mm spread diameter, whereas strain NU149 *gadE* had a 57.34 mm spread diameter that was significantly wider ( $P < 0.05$ ). Complementation of the *gadE* mutation brought the spread diameter back down to a wild-type level (7.00 mm). These results indicate that GadE also affects UPEC motility.

## DISCUSSION

The production of flagella in UPEC is vital for their pathogenesis in a human host, enabling the bacteria to ascend the urinary tract<sup>[4-7,11]</sup>. A transcriptome study of a UPEC strain in the murine urinary tract over time demonstrated that several genes that are involved in flagella biosynthesis and chemotaxis, including the *fliC* structural gene, had their transcription down-regulated in this environment<sup>[42]</sup>. Within the urinary tract, the *E. coli* encounter an environment that typically has a slightly acidic pH and osmotic changes that increase as the bacteria move into the kidneys of the host<sup>[14-16]</sup>. *E. coli* is able to survive in acidic pH environments that include the human and murine urinary tracts because of AR systems that include the glutamate decarboxylase system<sup>[15-18]</sup>. GadE is an important protein that regulates this AR system<sup>[31-33]</sup>. Since GadE is important for regulating genes in one AR system, could the GadE regulator of the glutamate decarboxylase AR system also be involved in the down-regulation of *fliC* in uropathogenic *E. coli* growing in the murine urinary tract?

To answer the question above, we designed a *fliC-lacZ* reporter system on a single copy number plasmid to measure *fliC* transcription within *E. coli* growing in various environments that might be encountered in the urinary tract. Our results showed *fliC* transcription fell in both *E. coli* strains grown in a pH 5.5 environment compared to a neutral pH environment, suggesting one or more proteins produced by *E. coli* growing in an acidic pH environment represses *fliC* transcription. A previous study revealed a substantial drop in motility by *E. coli* grown in an acidic environment *vs* a neutral pH environment<sup>[43]</sup> that correlates with our experimental observations in this study. Moreover, *E. coli* growth in a high salt concentration medium also caused repression of *fliC* transcription. Li *et al*<sup>[44]</sup> observed that *E. coli* grown in a high-

**Table 3** Effect of osmolarity on *fliC::lacZ* gene transcription in *Escherichia coli* grown in buffered pH 5.5 and 7.0 Luria Bertani media with different osmolarities

<i>E. coli</i> strain	NaCl (mmol/L)	Gal activity <sup>1</sup>	
		pH 5.5	pH 7.0
MC4100/pNK2-29	0	308 ± 104 <sup>2</sup>	1132 ± 130
MC4100/pNK2-29	100	338 ± 128	806 ± 41
MC4100/pNK2-29	200	251 ± 68.5	689 ± 173
MC4100/pNK2-29	400	62 ± 22.0	454 ± 71
NU149 LacZ1/pNK2-29	0	442 ± 72	1353 ± 98
NU149 LacZ1/pNK2-29	100	418 ± 61	976 ± 52
NU149 LacZ1/pNK2-29	200	293 ± 43	811 ± 75
NU149 LacZ1/pNK2-29	400	147 ± 39	489 ± 61

<sup>1</sup>Galactosidase activity measured as Miller units.<sup>2</sup>Data represents the mean ± standard deviation from three separate runs.

osmolarity medium were less motile compared to *E. coli* grown in a low-osmolarity medium.

A combination of pH changes and osmolarity changes was also examined using our *fliC-lacZ* system. In a low pH/high-osmolarity medium, the growing *E. coli* exhibited an additive level of repression of *fliC* transcription that is in line with the previous transcriptome study<sup>[42]</sup>.

Two environmental variables are at play in a low pH/high-osmolarity environment. To adapt to acidic pH conditions, *E. coli* rely on AR systems and their corresponding regulators, such as GadE. On the other hand, the OmpR-EnvZ two-component system is the main osmotic stress regulatory system in *E. coli*<sup>[45]</sup>. OmpR has been shown to regulate flagella expression<sup>[46,47]</sup> and is likely partially responsible for repressing *fliC* transcription in the high-osmolarity environment that we tested. Furthermore, OmpR-regulated genes are tied to the acid response in *E. coli* and *Salmonella enterica*<sup>[48,49]</sup>.

Since GadE is a central player in AR system regulation, we examined *fliC* transcription and motility in *gadE* mutant strains *vs* the wild-type strains. By deleting the *gadE* gene, *E. coli* *fliC* transcription was derepressed, particularly in *E. coli* growing in an acidic pH environment. Complementation of the *gadE* mutation with the *gadE* gene on a multicopy plasmid caused additional suppression of *fliC* transcription that was below wild-type levels. Furthermore, a  $\Delta$ *gadE* mutation in K-12 and UPEC strains led to significantly greater motility compared to the wild-type strain. Together, these data suggest that GadE represses *fliC* transcription either by directly binding to the *fliC* promoter to repress transcription or acting in an indirect manner by influencing expression of FlhD that in turn regulates *fliC*<sup>[50,51]</sup>. However, GadE does not appear to affect osmotic control of *fliC* transcription.

What would be the advantage of a loss of flagella expression in *E. coli* growing in the human kidney? Flagella protruding from the surface of *E. coli* cells represent a target of the host's immune system. Flagellated *E. coli* cells are more likely to be phagocytized than no-flagellated cells<sup>[52]</sup>. *E. coli* that have reached the kidneys would be in a low pH/high-osmolarity environment where the flagella are no longer needed and may in fact be a detriment to their survival. Through the regulatory effects of the GadE and OmpR proteins, *fliC* transcription may be shut down, causing the bacterial cells to lose their flagella and be able to hide behind their anti-phagocytic capsules.

**Table 4 Assessing a *gadE* and mutation and complementation on *fliC::lacZ* gene transcription in *Escherichia coli* grown in buffered pH 5.5 and 7.0 Luria Bertani media with different osmolarities**

<i>E. coli</i> strain	Gal activity <sup>1</sup>			
	pH 5.5	pH 5.5 <sup>2</sup>	pH 7.0	pH 7.0
EK227/pNK2-29	540 ± 51 <sup>3</sup>	165 ± 59	1520 ± 144	540 ± 66
EF1007/pNK2-29 <sup>4</sup>	1742 ± 109	470 ± 106	2196 ± 173	681 ± 135
EF1083/pNK2-29	295 ± 93	131 ± 20	794 ± 145	404 ± 41

<sup>1</sup>Galactosidase activity measured as Miller units.<sup>2</sup>400 mmol/L added NaCl.<sup>3</sup>Data represents the mean ± standard deviation from three separate runs.<sup>4</sup>EF1007 is *gadE* and EF1083 is *gadE*/pGadE+.**Table 5 Motility of *Escherichia coli* strains NU149 and EK227, their *gadE* mutants, and complemented *gadE* mutants grown in pH 5.5 Luria Bertani**

Strain	Motility (mm) <sup>1</sup>
NU149	10.67 ± 1.25 <sup>2</sup>
NU149 <i>gadE</i>	57.34 ± 10.21
NU149 <i>gadE</i> /pPCRScrip <i>gadE</i>	7.00 ± 0.82
EK227	8.33 ± 1.52
EF1007 ( <i>gadE</i> )	45.00 ± 2.00
EF1083 ( <i>gadE</i> /pPCRScrip <i>gadE</i> )	6.67 ± 1.53

<sup>1</sup>Spread diameter after 24 h on a motility plate measured in mm.<sup>2</sup>Data represents the mean ± standard deviation from three separate runs.

## ARTICLE HIGHLIGHTS

### Research background

Uropathogenic *Escherichia coli* (UPEC) is the number one cause of urinary tract infection in women. Motility driven by the action of flagella is critical for UPEC pathogenesis. How *Escherichia coli* (*E. coli*) adapts to a low pH/high osmolarity environment is essential for the species survival. Acid tolerance systems, such as the System two system, are important for UPEC survival in a low pH environment.

### Research motivation

Our key problem to be solved was whether GadE, a part of the acid response two system, regulates transcription of the *fliC* gene, and in turn, UPEC motility.

### Research objectives

Determine whether GadE regulated *fliC* transcription and subsequent motility of the *E. coli*.

### Research methods

We created a *fliC-lacZ* reporter system on a single-copy number plasmid and measured b-galactosidase levels in both a K-12 and UPEC clinical isolate. Furthermore, motility was assessed in both *E. coli* strains by inoculating wild-type, *gadE* mutant, and complemented *gadE* mutant strains onto motility agar.

### Research results

Transcription of *fliC* was significantly lower in *E. coli* grown in pH 5.5 Luria Bertani compared to pH 7.0 Luria Bertani. A mutation in the *gadE* gene led to higher *fliC* expression in that strain *vs* wild-type bacteria. Motility was significantly higher in the *gadE* mutant strain compared to the wild-type strain.

### Research conclusions

We confirmed that *fliC* transcription was down-regulated in *E. coli* grown in a low pH/high osmolarity environment compared to a neutral pH/low osmolarity environment. GadE appears to either directly or indirectly regulate *fliC* transcription in *E. coli*.

### Research perspectives

Future work could be done to affirm the GadE regulation of flagella expression in *E. coli*.

## ACKNOWLEDGEMENTS

The authors wish to thank John Foster for a critical reading of the manuscript. We wish to thank Linda Kenney (MC4100) and John Foster (EK227, EF1007, EF1083) for strains used in this study.

## REFERENCES

- 1 **Foxman B.** The epidemiology of urinary tract infection. *Nat Rev Urol* 2010; **7**: 653-660 [PMID: [21139641](#) DOI: [10.1038/nrurol.2010.190](#)]
- 2 **Foxman B.** Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infect Dis Clin North Am* 2014; **28**: 1-13 [PMID: [24484571](#) DOI: [10.1016/j.idc.2013.09.003](#)]
- 3 **Schappert SM, Rechtsteiner EA.** Ambulatory medical care utilization estimates for 2007. *Vital Health Stat 13* 2011; 1-38 [PMID: [21614897](#)]
- 4 **Lane MC, Alteri CJ, Smith SN, Mobley HL.** Expression of flagella is coincident with uropathogenic *Escherichia coli* ascension to the upper urinary tract. *Proc Natl Acad Sci USA* 2007; **104**: 16669-16674 [PMID: [17925449](#) DOI: [10.1073/pnas.0607898104](#)]
- 5 **Lane MC, Lockatell V, Monterosso G, Lamphier D, Weinert J, Hebel JR, Johnson DE, Mobley HL.** Role of motility in the colonization of uropathogenic *Escherichia coli* in the urinary tract. *Infect Immun* 2005; **73**: 7644-7656 [PMID: [16239569](#) DOI: [10.1128/IAI.73.11.7644-7656.2005](#)]
- 6 **Schwan WR.** Flagella allow uropathogenic *Escherichia coli* ascension into murine kidneys. *Int J Med Microbiol* 2008; **298**: 441-447 [PMID: [17888727](#) DOI: [10.1016/j.ijmm.2007.05.009](#)]
- 7 **Wright KJ, Seed PC, Hultgren SJ.** Uropathogenic *Escherichia coli* flagella aid in efficient urinary tract colonization. *Infect Immun* 2005; **73**: 7657-7668 [PMID: [16239570](#) DOI: [10.1128/IAI.73.11.7657-7668.2005](#)]
- 8 **Iino T.** Genetics of structure and function of bacterial flagella. *Annu Rev Genet* 1977; **11**: 161-182 [PMID: [339815](#) DOI: [10.1146/annurev.ge.11.120177.001113](#)]
- 9 **Jones CJ, Aizawa S.** The bacterial flagellum and flagellar motor: structure, assembly and function. *Adv Microb Physiol* 1991; **32**: 109-172 [PMID: [1882727](#) DOI: [10.1016/s0065-2911\(08\)60007-7](#)]
- 10 **Silverman M, Simon MI.** Bacterial flagella. *Annu Rev Microbiol* 1977; **31**: 397-419 [PMID: [410356](#) DOI: [10.1146/annurev.mi.31.100177.002145](#)]
- 11 **Haugen BJ, Pellett S, Redford P, Hamilton HL, Roesch PL, Welch RA.** In vivo gene expression analysis identifies genes required for enhanced colonization of the mouse urinary tract by uropathogenic *Escherichia coli* strain CFT073 dsdA. *Infect Immun* 2007; **75**: 278-289 [PMID: [17074858](#) DOI: [10.1128/IAI.01319-06](#)]
- 12 **De Souza GM, Neto ERDS, da Silva AM, Iacia MVMS, Rodrigues MVP, Cataneli Pereira V, Winkelstroter LK.** Comparative Study Of Genetic Diversity, Virulence Genotype, Biofilm Formation And Antimicrobial Resistance Of Uropathogenic *Escherichia coli* (UPEC) Isolated From Nosocomial And Community Acquired Urinary Tract Infections. *Infect Drug Resist* 2019; **12**: 3595-3606 [PMID: [31819543](#) DOI: [10.2147/IDR.S228612](#)]
- 13 **Zhao L, Gao S, Huan H, Xu X, Zhu X, Yang W, Gao Q, Liu X.** Comparison of virulence factors and expression of specific genes between uropathogenic *Escherichia coli* and avian pathogenic *E. coli* in a murine urinary tract infection model and a chicken challenge model. *Microbiology* 2009; **155**: 1634-1644 [PMID: [19372154](#) DOI: [10.1099/mic.0.024869-0](#)]
- 14 **Kunin CM, Chambers ST, Kass E, Svanborg Eden C.** Osmoprotective properties for bacteria of renal papilla and urine: role of betaines as osmoprotectant molecules. In: Kass E, Svanborg Eden C. Host-parasite interactions in urinary tract infections. Kass E, Svanborg Eden C. Illinois: Chicago, 1989: 327-332
- 15 **Loeb WF, Quimby FW.** The clinical chemistry of laboratory animals. Pergamon Press: New York, 1989
- 16 **Ross DL, Neely AE.** Textbook of urinalysis and bodily fluids. Virginia: Norwalk, 1983
- 17 **Foster JW.** *Escherichia coli* acid resistance: tales of an amateur acidophile. *Nat Rev Microbiol* 2004; **2**: 898-907 [PMID: [15494746](#) DOI: [10.1038/nrmicro1021](#)]
- 18 **Lin J, Lee IS, Frey J, Slonczewski JL, Foster JW.** Comparative analysis of extreme acid survival in *Salmonella typhimurium*, *Shigella flexneri*, and *Escherichia coli*. *J Bacteriol* 1995; **177**: 4097-4104 [PMID: [7608084](#) DOI: [10.1128/jb.177.14.4097-4104.1995](#)]
- 19 **Lin J, Smith MP, Chapin KC, Baik HS, Bennett GN, Foster JW.** Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. *Appl Environ Microbiol* 1996; **62**: 3094-3100 [PMID: [8795195](#)]
- 20 **Richard HT, Foster JW.** Acid resistance in *Escherichia coli*. *Adv Appl Microbiol* 2003; **52**: 167-186 [PMID: [12964244](#) DOI: [10.1016/s0065-2164\(03\)01007-4](#)]
- 21 **Li Z, Jiang B, Zhang X, Yang Y, Hardwidge PR, Ren W, Zhu G.** The role of bacterial cell envelope structures in acid stress resistance in *E. coli*. *Appl Microbiol Biotechnol* 2020; **104**: 2911-2921 [PMID: [32067056](#) DOI: [10.1007/s00253-020-10453-x](#)]
- 22 **Castanie-Cornet MP, Penfound TA, Smith D, Elliott JF, Foster JW.** Control of acid resistance in *Escherichia coli*. *J Bacteriol* 1999; **181**: 3525-3535 [PMID: [10348866](#)]
- 23 **Foster JW.** When protons attack: microbial strategies of acid adaptation. *Curr Opin Microbiol* 1999; **2**: 170-174 [PMID: [10322170](#) DOI: [10.1016/S1369-5274\(99\)80030-7](#)]
- 24 **Pennacchietti E, D'Alonzo C, Freddi L, Occhialini A, De Biase D.** The Glutaminase-Dependent Acid Resistance System: Qualitative and Quantitative Assays and Analysis of Its Distribution in Enteric Bacteria. *Front Microbiol* 2018; **9**: 2869 [PMID: [30498489](#) DOI: [10.3389/fmicb.2018.02869](#)]
- 25 **Chen L, Zhao X, Wu J, Liu Q, Pang X, Yang H.** Metabolic characterisation of eight *Escherichia coli* strains including "Big Six" and acidic responses of selected strains revealed by NMR spectroscopy. *Food Microbiol* 2020; **88**: 103399 [PMID: [31997756](#) DOI: [10.1016/j.fm.2019.103399](#)]
- 26 **De Biase D, Pennacchietti E.** Glutamate decarboxylase-dependent acid resistance in orally acquired bacteria: function, distribution and biomedical implications of the gadBC operon. *Mol Microbiol* 2012; **86**: 770-786 [PMID: [22995042](#) DOI: [10.1111/mmi.12020](#)]
- 27 **De Biase D, Tramonti A, John RA, Bossa F.** Isolation, overexpression, and biochemical characterization of the two isoforms of glutamic acid decarboxylase from *Escherichia coli*. *Protein Expr Purif* 1996; **8**: 430-

- 438 [PMID: 8954890 DOI: 10.1006/prev.1996.0121]
- 28 **Hersh BM**, Farooq FT, Barstad DN, Blankenhorn DL, Slonczewski JL. A glutamate-dependent acid resistance gene in *Escherichia coli*. *J Bacteriol* 1996; **178**: 3978-3981 [PMID: 8682809 DOI: 10.1128/jb.178.13.3978-3981.1996]
  - 29 **Masuda N**, Church GM. Regulatory network of acid resistance genes in *Escherichia coli*. *Mol Microbiol* 2003; **48**: 699-712 [PMID: 12694615 DOI: 10.1046/j.1365-2958.2003.03477.x]
  - 30 **Smith DK**, Kassam T, Singh B, Elliott JF. *Escherichia coli* has two homologous glutamate decarboxylase genes that map to distinct loci. *J Bacteriol* 1992; **174**: 5820-5826
  - 31 **Hommais F**, Krin E, Coppée JY, Lacroix C, Yeramian E, Danchin A, Bertin P. GadE (YhiE): a novel activator involved in the response to acid environment in *Escherichia coli*. *Microbiology* 2004; **150**: 61-72 [PMID: 14702398 DOI: 10.1099/mic.0.26659-0]
  - 32 **Castanié-Cornet MP**, Foster JW. *Escherichia coli* acid resistance: cAMP receptor protein and a 20 bp cis-acting sequence control pH and stationary phase expression of the *gadA* and *gadBC* glutamate decarboxylase genes. *Microbiology* 2001; **147**: 709-715 [PMID: 11238978 DOI: 10.1099/00221287-147-3-709]
  - 33 **Ma Z**, Gong S, Richard H, Tucker DL, Conway T, Foster JW. GadE (YhiE) activates glutamate decarboxylase-dependent acid resistance in *Escherichia coli* K-12. *Mol Microbiol* 2003; **49**: 1309-1320 [PMID: 12940989 DOI: 10.1046/j.1365-2958.2003.03633.x]
  - 34 **Castanié-Cornet MP**, Treffandier H, Francez-Charlot A, Gutierrez C, Cam K. The glutamate-dependent acid resistance system in *Escherichia coli*: essential and dual role of the His-Asp phosphorelay RcsCDB/AF. *Microbiology* 2007; **153**: 238-246 [PMID: 17185552 DOI: 10.1099/mic.0.29278-0]
  - 35 **Castanié-Cornet MP**, Cam K, Bastiat B, Cros A, Bordes P, Gutierrez C. Acid stress response in *Escherichia coli*: mechanism of regulation of *gadA* transcription by RcsB and GadE. *Nucleic Acids Res* 2010; **38**: 3546-3554 [PMID: 20189963 DOI: 10.1093/nar/gkq097]
  - 36 **Schaeffer AJ**, Schwan WR, Hultgren SJ, Duncan JL. Relationship of type 1 pilus expression in *Escherichia coli* to ascending urinary tract infections in mice. *Infect Immun* 1987; **55**: 373-380 [PMID: 2879794]
  - 37 **de Lorenzo V**, Herrero M, Jakubzik U, Timmis KN. Mini-Tn5 transposon derivatives for insertion mutagenesis, promoter probing, and chromosomal insertion of cloned DNA in gram-negative eubacteria. *J Bacteriol* 1990; **172**: 6568-6572 [PMID: 2172217 DOI: 10.1128/jb.172.11.6568-6572.1990]
  - 38 **Schwan WR**, Lee JL, Lenard FA, Matthews BT, Beck MT. Osmolarity and pH growth conditions regulate *fim* gene transcription and type 1 pilus expression in uropathogenic *Escherichia coli*. *Infect Immun* 2002; **70**: 1391-1402 [PMID: 11854225 DOI: 10.1128/iai.70.3.1391-1402.2002]
  - 39 **Miller JH**. Experiments in molecular genetics. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1972
  - 40 **Datsenko KA**, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA* 2000; **97**: 6640-6645 [PMID: 10829079 DOI: 10.1073/pnas.120163297]
  - 41 **Craven RC**, Montie TC. Motility and chemotaxis of three strains of *Pseudomonas aeruginosa* used for virulence studies. *Can J Microbiol* 1981; **27**: 458-460 [PMID: 6786721 DOI: 10.1139/m81-070]
  - 42 **Snyder JA**, Haugen BJ, Buckles EL, Lockett CV, Johnson DE, Donnenberg MS, Welch RA, Mobley HL. Transcriptome of uropathogenic *Escherichia coli* during urinary tract infection. *Infect Immun* 2004; **72**: 6373-6381 [PMID: 15501767 DOI: 10.1128/IAI.72.11.6373-6381.2004]
  - 43 **Adler J**, Templeton B. The effect of environmental conditions on the motility of *Escherichia coli*. *J Gen Microbiol* 1967; **46**: 175-184 [PMID: 4961758 DOI: 10.1099/00221287-46-2-175]
  - 44 **Li C**, Louise CJ, Shi W, Adler J. Adverse conditions which cause lack of flagella in *Escherichia coli*. *J Bacteriol* 1993; **175**: 2229-2235 [PMID: 8385664 DOI: 10.1128/jb.175.8.2229-2235.1993]
  - 45 **Igo MM**, Slauch JM, Silhavy TJ. Signal transduction in bacteria: kinases that control gene expression. *New Biol* 1990; **2**: 5-9 [PMID: 1964084]
  - 46 **Shin S**, Park C. Modulation of flagellar expression in *Escherichia coli* by acetyl phosphate and the osmoregulator OmpR. *J Bacteriol* 1995; **177**: 4696-4702 [PMID: 7642497 DOI: 10.1128/jb.177.16.4696-4702.1995]
  - 47 **Prüss BM**, Wolfe AJ. Regulation of acetyl phosphate synthesis and degradation, and the control of flagellar expression in *Escherichia coli*. *Mol Microbiol* 1994; **12**: 973-984 [PMID: 7934904 DOI: 10.1111/j.1365-2958.1994.tb01085.x]
  - 48 **Bang IS**, Audia JP, Park YK, Foster JW. Autoinduction of the *ompR* response regulator by acid shock and control of the *Salmonella enterica* acid tolerance response. *Mol Microbiol* 2002; **44**: 1235-1250 [PMID: 12068808 DOI: 10.1046/j.1365-2958.2002.02937.x]
  - 49 **Chakraborty S**, Kenney LJ. A New Role of OmpR in Acid and Osmotic Stress in *Salmonella* and *E. coli*. *Front Microbiol* 2018; **9**: 2656 [PMID: 30524381 DOI: 10.3389/fmicb.2018.02656]
  - 50 **Prüß BM**. Involvement of Two-Component Signaling on Bacterial Motility and Biofilm Development. *J Bacteriol* 2017; **199**: e00259-17 [PMID: 28533218 DOI: 10.1128/JB.00259-17]
  - 51 **Prüss BM**, Besemann C, Denton A, Wolfe AJ. A complex transcription network controls the early stages of biofilm development by *Escherichia coli*. *J Bacteriol* 2006; **188**: 3731-3739 [PMID: 16707665 DOI: 10.1128/JB.01780-05]
  - 52 **Sharon N**, Eshdat Y, Silverblatt FJ, Ofek I. Bacterial adherence to cell surface sugars. *Ciba Found Symp* 1981; **80**: 119-141 [PMID: 6114817 DOI: 10.1002/9780470720639.ch9]
  - 53 **McCann MP**, Kidwell JP, Matin A. The putative sigma factor KatF has a central role in development of starvation-mediated general resistance in *Escherichia coli*. *J Bacteriol* 1991; **173**: 4188-4194 [PMID: 2061293 DOI: 10.1128/jb.173.13.4188-4194.1991]
  - 54 **Tucker DL**, Tucker N, Conway T. Gene expression profiling of the pH response in *Escherichia coli*. *J Bacteriol* 2002; **184**: 6551-6558 [PMID: 12426343 DOI: 10.1128/jb.184.23.6551-6558.2002]



Published By Baishideng Publishing Group Inc  
7041 Koll Center Parkway, Suite 160 Pleasanton, CA 94566, USA  
Telephone: +1-925-3991568  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <https://www.f6publishing.com/helpdesk>  
<https://www.wjgnet.com>

