J. Microbiol. Biotechnol. (2014), 24(3), 401–407
http://dx.doi.org/10.4014/jmb.1310.10091

jmb

Interaction Between the Quorum Sensing and Stringent Response Regulation Systems in the Enterohemorrhagic *Escherichia coli* O157:H7 EDL933 Strain

Kyung-Hwan Oh and Seung-Hak Cho*

Division of Enteric Diseases, Center for Infectious Diseases, Korea National Institute of Health, Osong 363-951, Republic of Korea

Received: October 25, 2013
Revised: November 28, 2013
Accepted: December 5, 2013

First published online
December 9, 2013

**Corresponding author*
Phone: +82-43-719-8112;
Fax: +82-43-719-8149;
E-mail: skcho38@korea.kr

Supplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872

Quorum sensing and the stringent response are well-known regulation systems for the expression of virulence genes in enterohemorrhagic *Escherichia coli* (EHEC). However, how these two systems interact is not well known. *E. coli* strains with mutations in two regulation systems, Δ*luxS* (ECM101) and Δ*luxS*Δ*relA*Δ*spoT* (ECM201), and the Δ*luxS* complement strain to ECM201 (ECM202) were created from EHEC O157:H7 EDL933 to investigate how the regulatory systems interact. The phenotypic changes of the mutant strains were characterized and compared with the wild type. The mutant strains exhibited no obvious growth defects, although acid resistance and cellular cytotoxicity were decreased significantly in all the mutant strains. Phenotypic characterization revealed that mutations in the stringent response system (ECM201 and ECM202) influenced the metabolic (defective utilization of arabinose and L-sorbose) and enzymatic activities (decreased trypsin activity, and increased α-glucosidase activity). In contrast, the quorum sensing system mutant (ECM101) did not display these phenotypes. The motility of the quorum sensing system mutant (ECM101) was unchanged, but mutation in the stringent response system influenced the motility. Our results suggest that quorum sensing interacts with the stringent response regulation system.

Keywords: Quorum sensing, stringent response, EHEC O157:H7 EDL933, phenotypic characterization

Introduction

Outbreaks of enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 in humans were first reported in 1983 [15, 22, 31]. Since then, EHEC has been recognized as an important foodborne pathogen that causes hemorrhagic colitis and hemolytic uremic syndrome [4, 20]. EHEC strains express Shiga toxins (Stx), cause attaching and effecting lesions on epithelial cells, and possess an ca. 60 MDa plasmid [20].

Quorum sensing is a mechanism of cell-to-cell signaling involving the production of autoinducers (AIs), which are hormone-like compounds [25]. When they reach a certain concentration threshold, they interact with bacterial regulatory proteins to regulate gene expression [24]. Specifically, AI-2 and -3, which mediate quorum sensing signaling, have been extensively studied in EHEC [19, 29]. In addition, LuxS is responsible for the production of AI-2 [5], and is indirectly involved in the production of AI-3 [16, 25, 30].

Bacterial cells encounter various environmental stresses, and make appropriate adjustments to ensure survival [1, 3, 21]. The stringent response is a stress response that is important for bacterial survival under conditions of stress, including amino acid starvation, fatty acid limitations, lack of iron, heat shock, and low pH [3, 27]. Stringent response signaling is achieved by the accumulation of the effector nucleotides, guanosine tetra- and pentaphosphate (ppGpp and pppGpp) [21], of which ppGpp is the predominant and more stable of the two species. Two enzymes, RelA and SpoT, regulate the levels of ppGpp. RelA is the major synthetase of ppGpp under stressful conditions, whereas SpoT is a bifunctional enzyme with weak synthetase activity of (p)ppGpp, and hydrolase activity for degrading

(p)ppGpp [11]. As such, Δ*relA spoT* mutants are unable to produce (p)ppGpp and have multiple defects, including auxotrophies for several amino acids [32].

The quorum sensing and stringent response systems are known to regulate gene expression in various bacteria [20]. Previous studies showed that the *luxS* mutation can cause global changes in the expression of many genes that are involved in biochemical and metabolic cellular processes [12, 14, 23, 24, 25, 28, 29]. Other studies demonstrated that *relA* and *spoT* mutations in bacterial species, including *Yersinia* and *Enterococcus*, caused growth defects compared with wild type [1, 26]. However, how these two systems interact remains unclear. We therefore assessed the interaction between these systems in EHEC O157:H7 EDL933.

Materials and Methods

Strains, Media, and Growth Conditions

Bacterial strains and plasmids used in this study are listed in Table 1. All cultures were grown at 37°C on Luria-Bertani agar plates (BD, Sparks, USA), and aerobically at 37°C with shaking at 200 rpm in LB broth. To confirm the mutations in the stringent response system, all strains were grown at 37°C in 1–50% LB medium, giving a minimum concentration of 5% LB. In this study, we therefore used both standard and 5% LB medium for studies of bacterial growth, acid resistance, cell cytotoxicity, and motility (Supplementary data, Tables S1–S5). Antibiotics were used at the following concentrations: ampicillin (Sigma, USA, 100 μg/ml), kanamycin (Sigma, 50 μg/ml), and chloramphenicol (Sigma, 24 μg/ml).

Construction of Deletion Mutants and the Complementation Strain

An isogenic Δ*luxS* mutant lacking the entire structural *luxS* gene was constructed using a one-step inactivation method [9, 18]. A linear DNA fragment harboring the kanamycin-resistant gene *kan* and homologous *luxS*-flanking sequences was amplified using the gene-specific primers LuxS-kan-F and LuxS-kan-R, as shown in Table 2, and the pKD13 plasmid as the template. The fragment was introduced into EDL933 competent cells by electroporation (GenePulser Xcell; BIO-RAD Laboratories, Hercules, CA, USA), and transformants were selected on LB agar plates containing kanamycin. The *kan* gene was then removed by recombination using ELP recombinase. The deletion of *luxS* was confirmed by PCR, using *luxS*-specific primers (Fig. 1). For the stringent response mutant, the two donor strains S17-λ pir (pCVD442/Δ*rel*A::*aph*A-3) and S17-λ pir (pCVD442/Δ*spo*T::cat) were a generous gift from Jang W. Yoon at Seoul National University (J.W. Yoon and J.B. Kaper, unpublished). A triple mutant (Δ*luxS*Δ*relA*Δ*spoT*) was then constructed using the quorum sensing mutant (Δ*luxS*) as a recipient by conjugation, as described previously [10]. All donor and recipient strains were grown to the late exponential phase, washed in LB, and mixed at a 1:10 ratio. Mating mixtures were resuspended in 50 μl of LB, and plated on to LB agar plates. After incubation at 37°C for 12 h, they were resuspended by vortexing, and diluted in liquid medium. Transconjugants were then selected on LB plates supplemented with the appropriate antibiotics [8]. For the construction of the complementation strain, an isopropyl-β-D-thiogalactopyranoside-inducible expression plasmid carrying the intact *luxS* gene (pEXP5-CT/*luxS*) was created, and transformed into the mutant strain ECM201. Expression of *luxS* was then induced using 10 mM IPTG.

Table 1. Bacterial strains and plasmids used in this study.

Strain/plasmid	Relevant genotype	Reference
Strains		
EDL933	Wild-type strain, ATCC43895	[20]
S17-1 λ pir	Donor strain	[22]
ECM100	EDL933 *luxS*::kan	This study
ECM101	EDL933 Δ*luxS*	This study
ECM201	EDL933 Δ*luxS* Δ*relA* Δ*spoT*	This study
ECM202	ECM201 containing pEXP5-CT/*luxS*	This study
Plasmids		
pKD13	Template plasmid carrying a KmR cassette (ApR, KmR)	[12]
pKD46	Arabinose-inducible lambda red recombinase expression plasmid (ApR)	[12]
pCP20	Temperature-sensitive plasmid carrying a FLP flipase (ApR, CmR)	[12]
pEXP5-CT	IPTG-inducible C-terminal polyhistidine (6× His) expression vector (ApR)	Invitrogen
pEXP-CT/*luxS*	pEXP5-CT carrying the intact *luxS* gene from CI03J (ApR)	This study

Table 2. Primers used in this study.

Primers	Primer sequence (5′–3′)	Size (bp)
LuxS	GGATGACGCAACAGCAGG	967
	CGCGAGGCGTCTGAACGC	
LuxS-kan	GCGGTGCGCACTAAGTACAACTAAGCCAGTTCATTTGGTGTAGGCTGGAGCTGCTTC	1,403
	TGCGGTGTGGCTGGAAAAACACGCCTGACAGAAAAGATTCCGGGGATCCGTCGACC	
relA	CTGGCGATGCTGGATATGTT	2,200
	TGAGCGCCTGCATTAACGTA	
spoT	CTATTGCTGAGGGTCGTCGT	2,100
	CATGCAGACGGTCAGATCAG	

Bacterial Growth Test

The wild-type and mutant strains were grown aerobically at 37°C with shaking at 200 rpm in LB broth. Bacterial growth was then monitored every hour by measuring the optical density at 600 nm (OD_{600}). At least three independent experiments were performed, and the data are presented as the mean ± standard error (SE). Viable cell counts were calculated after serial dilution in 0.85% saline and plating on Tryptic Soy Agar (BD, Sparks, USA) plates [2].

Acid Resistance Assay

The acid resistance of bacteria was assessed by inoculating 30 ml of LB broth with a 1:1,000 dilution of cultures at pH 7.0, 2.5, and 1.5, and incubating for 8 h. After incubation for 2 h, the colony-forming units (CFU) of the cultures were counted [6].

Carbohydrate Utilization and Enzyme Activity Tests

The ability of the strains to grow with carbon sources were assessed using the API 50CH (API system; bioMerieux, Paris, France) standardized system, following the manufacturer's instructions [17]. Enzyme activities were measured using the API ZYM (API system; bioMerieux) [13].

Cytotoxicity Assay

To assess cytotoxicity, the dehydrogenase activity in the culture medium was assessed using a cytotoxicity detection kit (EZ-Cytox; Daeillab Service Co. Ltd, Seoul, Korea). Vero cells cultured in DMEM (Dulbecco's modified Eagle's medium; Gibco, Grand Island, USA) were plated onto 96-well tissue culture plates at a density of 1×10^4 cells per well, and incubated at 37°C for 24 h in the presence of 5% CO_2. The cytotoxicity of the EHEC O157:H7 EDL933 and mutant strains was determined by treating cells with 10 µl of bacterial culture supernatant for 8 h. After incubation for 3 h at 37°C, the absorbance of each well at 450 nm was measured, with a reference wavelength at 650 nm (Molecular Devices, US/VERSAmax, Sunnyvale, USA).

Motility Assay

To investigate bacterial motility, EHEC O157:H7 EDL933 and mutant strains were stabbed into motility agar plates (1% tryptone, 0.25% NaCl, and 0.3% agar) and incubated at 37°C. The diameter of the motility halos was then measured after 10 h. The experiment

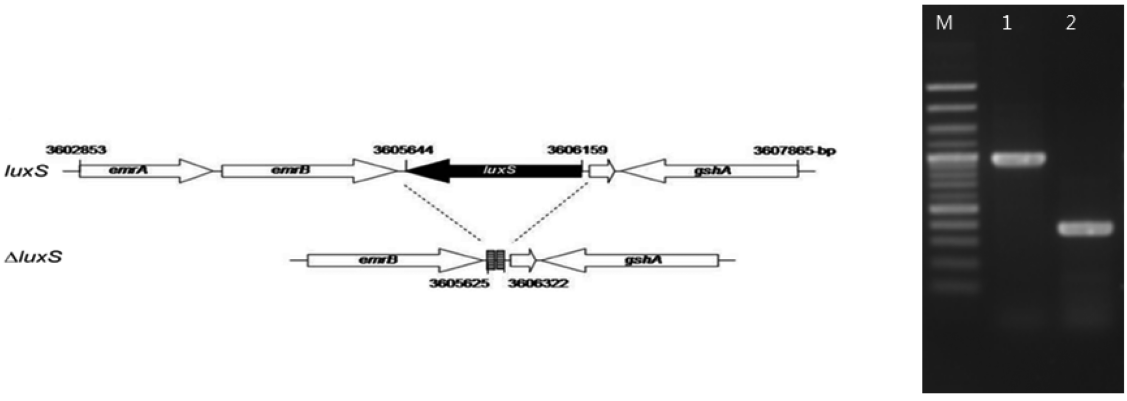

Fig. 1. Genetic mutation of the *luxS* gene in enterohemorrhagic *E. coli* O157:H7 EDL933.
PCR products were separated on 1.5% agarose gel, and the deletion of *luxS* was confirmed by PCR using gene-specific primers adjacent to *luxS*. Lane M = 1.0 kb plus DNA ladder; lane 1 = EDL933 (wild type strain); lane 2 = ECM101 (EDL933 Δ*luxS*).

was repeated using three independent cultures.

Results and Discussion

Construction of Deletion Mutants and Complemented Strain

To investigate the interaction of quorum sensing and stringent response regulation, single and triple mutant strains were constructed from EHEC O157:H7 EDL933 (Table 1). The resulting Δ*luxS* mutant strain was designated ECM101. The stringent response mutant to ECM101 (Δ*luxS*Δ*relA*Δ*spoT*) was constructed by conjugation, and designated ECM201. Finally, the Δ*luxS* complemented strain was constructed by the insertion of pEXP-CT/*luxS* to ECM201 and designated ECM202.

Table 3. The carbohydrate metabolism of EDL933 and mutant strains ECM101 (Δ*luxS* mutant), ECM201 (Δ*luxS*Δ*relA*Δ*spoT* mutant), and ECM202 (ECM201 containing pEXP5-CT/*luxS*).

Test	EDL933	ECM101	ECM201	ECM202
Control	–[a]	–	–	–
Glycerol	++	++	+	+
Erythritol	–	–	–	–
D-Arabinose	+	+	–	–
L-Arabinose	++	++	++	++
D-Ribose	++	++	++	++
D-Xylose	++	++	++	++
L-Xylose	–	–	–	–
D-Adonitol	–	–	–	–
Methyl-β D-xylopyranoside	–	–	–	–
D-Galactose	++	++	++	++
D-Glucose	++	++	++	++
D-Fructose	++	++	++	++
D-Mannose	++	++	++	++
L-Sorbose	+	++	–	–
L-Rhamnose	++	++	++	++
Dulcitol	++	++	++	++
Inositol	–	–	–	–
D-Mannitol	++	++	++	++
D-Sorbitol	–	–	–	–
Methyl-α D-mannopyranoside	–	–	–	–
Methyl-α D-glucopyranoside	–	–	–	–
N-acetylglucosamine	++	++	++	++
Amygdalin	–	–	–	–
Arbutin	–	–	–	–

[a]– = no utilization of the carbohydrate; + = good utilization; ++ = very good utilization.

Effects of Quorum Sensing and Stringent Response Systems on Metabolic Activities

To assess whether the mutations in quorum sensing and stringent response systems influenced the metabolic and enzyme activities in EHEC O157:H7 EDL933, the bacterial growth of wild-type and mutant strains was monitored by measuring ODs and counting viable cells. All the mutant strains showed similar growth rates to the wild-type strain in LB medium, suggesting that there were no obvious growth defects attributable to the mutations. However, the stringent response system mutation (in ECM201 and ECM202) affected both metabolic and enzymatic activities, whereas the quorum sensing system mutant strain (ECM101) had no effect on either metabolic or enzymatic activity. Specifically, the ECM101 strain showed the same metabolic activity pattern as wild type, whereas ECM201 and ECM202 showed defects in the ability to utilize D-arabinose and L-sorbose (Table 3). This is consistent with

Table 4. Enzymatic activity of EDL933 and mutant strains ECM101 (Δ*luxS* mutant), ECM201 (Δ*luxS*Δ*relA*Δ*spoT* mutant), and ECM202 (ECM201 containing pEXP5-CT/*luxS*).

Enzyme	Effect			
	EDL933	ECM101	ECM201	ECM202
Control	–[a]	–	–	–
Alkaline phosphatase	+++	+++	+++	+++
Esterase (C4)	–	–	–	–
Esterase lipase (C8)	–	–	–	–
Lipase (C14)	–	–	–	–
Leucine arylamidase	+++	+++	+++	+++
Valine arylamidase	–	–	–	–
Crystine arylamidase	–	–	+	-
Trypsin	+	+	–	–
α-Chymotrypsin	–	–	–	–
Acid phospatase	+++	+++	+++	+++
Naphtol-AS-BI-phosphohydrolase	++	++	++	++
α-Galactosidase	–	–	–	–
β-Glucuronidase	+++	+++	+++	+++
α-Glucosidase	–	–	++	+
β-Glucosidase	–	–	–	–
N-Acetyl-β-glucosaminidase	–	–	–	–
α-Mannosidase	–	–	–	–
α-Fucosidase	–	–	–	–

[a]– = no activity; + = low activity; ++ = medium activity; +++ = high activity.

a previous report, where a Δ*luxS* mutant of a clinical EHEC O157:H7 strain isolated in Korea was unable to utilize L-sorbose as a single carbon source [18]. Other studies suggested that some enteric bacterial pathogens might use their ability to degrade L-sorbose as their pathomechanism [7]. A defective ability to utilize L-sorbose therefore suggests that the stringent response system may have more correlation with the pathomechanism than the quorum sensing system. When enzymatic activities were compared, ECM201 and ECM202 exhibited different activities to wild type and ECM101. As shown in Table 4, trypsin activity was decreased and α-glucosidase activity was increased in ECM201 and ECM202 compared with wild type and ECM101. Interestingly, the cystine arylamidase activity of ECM201 was recovered, whereas wild type, ECM101, and ECM202 all lacked cystine arylamidase activity (Table 4).

Changes in Acid Resistance and Cell Cytotoxicity of the Mutant Strains

To assess the effect of the mutations on acid resistance, we measured the survival ratio of wild-type and mutant strains under low pH conditions. After incubation for 2 h, the survival ratio of wild type was 69% at pH 2.5 and 60% at pH 1.5. However, the mutant strains all had poor survival at both pH 2.5 and 1.5 (Fig. 2). To determine whether mutations in quorum sensing and stringent response systems influenced cellular cytotoxicity, we carried out an LDH test of wild-type and mutant strains using Vero cells. Although the cytotoxicity of all mutant strains was lower than wild type, the effects were more pronounced in ECM201 and ECM202 than in ECM101 (Fig. 3).

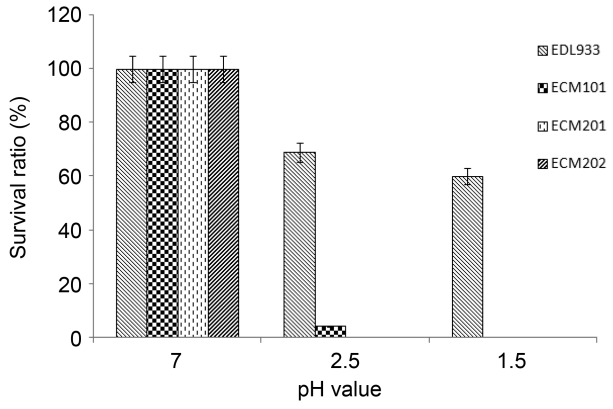

Fig. 2. The survival ratio of EDL933 and mutant strains ECM101 (Δ*luxS* mutant), ECM201 (Δ*luxS*Δ*relA*Δ*spoT* mutant), and ECM202 (ECM201 containing pEXP5-CT/*luxS*).
All strains were precultured in LB medium and then incubated in low pH LB.

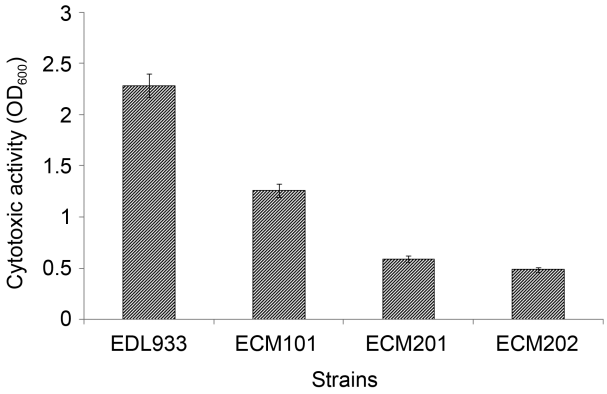

Fig. 3. Cytotoxic activity of the culture supernatants from the wild-type, ECM101 (Δ*luxS* mutant), ECM201 (Δ*luxS*Δ*relA*Δ*spoT* mutant), and ECM202 (ECM201 containing pEXP5-CT/*luxS*) strains.

Changes in the Motility of Bacterial Cells by Interaction Between the Two Regulation Systems

To compare the motility of the wild-type and mutant strains, bacterial swarm motility was assessed. The motility of the quorum sensing system mutant (ECM101) was unchanged, and was similar to wild type (Fig. 4). Interestingly, however, quorum sensing influenced the motility of the stringent response system. The motility of the stringent

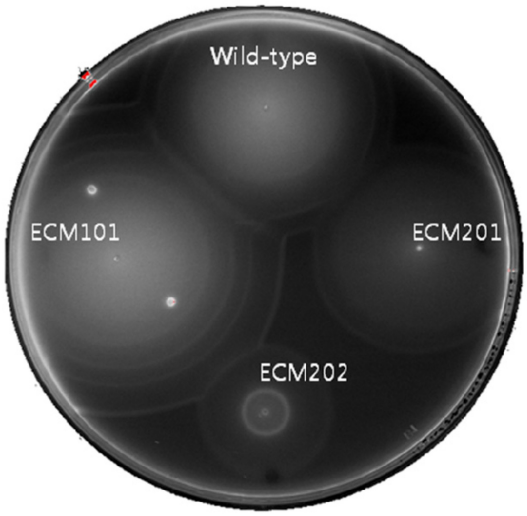

Fig. 4. The swarm motility of wild-type, ECM101 (Δ*luxS* mutant), ECM201 (Δ*luxS*Δ*relA*Δ*spoT* mutant), and ECM202 (ECM201 containing pEXP5-CT/*luxS*) strains in 0.3% tryptone agar after a 10 h incubation.

response mutant was decreased (data not shown), but the motility of the quorum sensing system and stringent response mutant (ECM201) was recovered to the levels of wild type. This result was confirmed by *luxS* complemented strain (ECM202) (Fig. 4).

In summary, our data suggest that quorum sensing interacts with the stringent response regulation system. However, further studies are necessary to define the mechanisms and genes responsible for the phenotypic changes.

Acknowledgments

This study was supported by the Korea National Institute of Health (NIH 4800-4845-300 and NIH 4800-4847-300 to S.H.C.). We thank Dr. Jang W. Yoon at Seoul National University for his kind gift of the two donor strains.

References

1. Abranches J, Martinez AR, Kajfasz JK, Chavez V, Garsin DA, Lemos JS. 2009. The molecular alarmone (p)ppGpp mediates stress responses, vancomycin tolerance, and virulence in *Enterococcus faecalis*. *J. Bacteriol.* **191:** 2248-2256.
2. Bansal T, Englert D, Lee JT, Hegde M, Wood TK, Jayaraman A. 2007. Differential effects of epinephrine, norepinephrine and indole on *Escherichia coli* O157:H7 chemotaxis, colonization and gene expression. *Infect. Immun.* **75:** 4597-4607.
3. Battesti A, Bouveret E. 2006. Acyl carrier protein/SpoT interaction, the switch linking SpoT-dependent stress response to fatty acid metabolism. *Mol. Microbiol.* **62:** 1048-1063.
4. Caprioli A, Morabito S, Brugere H, Oswald E. 2005. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Vet. Res.* **36:** 289-311.
5. Chen X, Schauder S, Potier N, Dorsselaer AV, Pelczer I, Bassler BL, Hughson FM. 2002. Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* **415:** 545-549.
6. Coldewey SM, Hartmann M, Schmidt DS, Engelking U, Ukena SN, Gunzer F. 2007. Impact of the *rpoS* genotype for acid resistance patterns of pathogenic and probiotic *Escherichia coli*. *BMC Microbiol.* **7:** 21.
7. Da Re S, Ghigo JM. 2006. A CsgD-independent pathway for cellulose production and biofilm formation in *Escherichia coli*. *J. Bacteriol*. **188:** 3073-3087.
8. Daniel PM, de la Fernando C. 2009. *Escherichia coli* genes affecting recipient ability in plasmid conjugation: are there any? *BMC Genomics* **10:** 71.
9. Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA* **97:** 6640-6645.
10. Donnenberg MS, Kaper JB. 1991. Construction of an *eae* deletion mutant of enteropathogenic *Escherichia coli* by using a positive-selection suicide vector. *Infect. Immun.* **59:** 4310-4317.
11. Gentry DR, Cashel M. 1996. Mutational analysis of the *Escherichia coli spo*T gene identifies distinct but overlapping regions involved in ppGpp synthesis and degradation. *Mol. Microbiol*. **19:** 1373-1384.
12. He YW, Zhang LH. 2008. Quorum sensing and virulence regulation in *Xanthomonas campestris*. *FEMS Microbiol. Rev*. **32:** 842-857.
13. Humble MW, King A, Phillips I. 1977. API ZYM: a simple rapid system for the detection of bacterial enzymes. *J. Clin. Pathol*. **30:** 275-277.
14. Jayaraman A, Wood TK. 2008. Bacterial quorum sensing: signals, circuits, and implications for biofilms and disease. *Annu. Rev. Biomed. Eng*. **10:** 145-167.
15. Karmali MA, Steele BT, Petric M, Lim C. 1983. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet* **1:** 619-620.
16. Kendall MM, Rasko DA, Sperandio V. 2007. Global effects of the cell-to-cell signaling molecules autoinducer-2, autoinducer-3, and epinephrine in a *luxS* mutant of enterohemorrhagic *Escherichia coli*. *Infect. Immun.* **75:** 4875-4884.
17. Kerr KG, Rotowa NA, Hawkey PM, Lacey RW. 1990. Evolution of the Mast ID and API 50CH systems for identification of *Listeria* spp. *Appl. Environ. Microbiol.* **56:** 657-660.
18. Kim JC, Yoon JW, Kim JB, Oh KH, Park MS, Lee BK, and Cho SH. 2010. Omics-based analysis of the *lux*S mutation in a clinical isolate of *Escherichia coli* O157:H7 in Korea. *J. Microbiol. Biotechnol*. **20:** 415-419.
19. Mellies JL, Barron AMS, Carmona AM. 2007. Enteropathogenic and enterohemorrhagic *Escherichia coli* virulence gene regulation. *Infect. Immun.* **75:** 4199-4210.
20. Nataro JP, Kaper JB. 1998. Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.* **11:** 142-201.
21. Potrykus K, Cashel M. 2008. (p)ppGpp: still magical? *Annu. Rev. Microbiol.* **62:** 35-51.
22. Riley LW, Remis RS, Helgerson SD, Mcgee HB, Wells JG, Davis BR, *et al*. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* **308:** 681-685.
23. Senadheera D, Cvitkovitxh DG. 2008. Quorum sensing and biofilm formation by *Streptococcus mutans*. *Adv. Exp. Med. Biol*. **631:** 178-188.
24. Sperandio V, Mellies JL, Nguyen W, Shin S, Kaper JB. 1999. Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **96:** 15196-15201.
25. Sperandio V, Torres AG, Giron BJA, Kaper JB. 2001.

Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157: H7. *J. Bacteriol.* **183:** 5187-5197.

26. Sun W, Roland KL, Branger CG, Kuang X, Curtiss R. 2009. The role of *relA* and *spoT* in *Yersinia pestis* KIM5$^+$ pathogenicity. *PLoS ONE* **4:** e6720.
27. Vinella D, Albrecht C, Cashel M, D'Ari R. 2005. Iron limitation induces SpoT-dependent accumulation of ppGpp in *Escherichia coli*. *Mol. Microbiol.* **56:** 958-970.
28. von Bodman SB, Willey JM, Diggle SP. 2008. Cell-cell communication in bacteria: united we stand. *J. Bacteriol*. **190:** 4377-4391.
29. Walters M, Sperandio V. 2006. Quorum sensing in *Escherichia coli* and *Salmonella*. *Int. J. Med. Microbiol*. **296:** 125-131.
30. Walters M, Sircili MP, Sperandio V. 2006. AI-3 synthesis is not dependent on *lux*S in *Escherichia coli*. *J. Bacteriol.* **188:** 5668-5681.
31. Wells JG, Davis BR, Wachsmuth IK, Riley LW, Remis RS, Sokolow R, Morris GK. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *J. Clin. Microbiol*. **18:** 512-520.
32. Xiao H, Kalman M, Ikehara K, Zemel S, Glaser G, Cashel M. 1991. Residual guanosine 3,5-bispyrophosphate synthetic activity of *relA* null mutants can be eliminated by *spoT* null mutations. *J. Biol. Chem*. **266:** 5980-5990.