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Antipathogenic Properties of Green Tea Polyphenol Epigallocatechin Gallate at Concentrations below the MIC against Enterohemorrhagic *Escherichia coli* O157:H7

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ABSTRACT

The inhibitory effects of green tea polyphenol epigallocatechin gallate (EGCG) on virulence phenotypes and gene expression regulated by quorum sensing (QS) in *Escherichia coli* O157:H7 were demonstrated at concentrations of 1 to 100 μg/ml, which are lower than the MIC (539 ± 22 μg/ml). At 25 μg/ml, the growth rate was not affected, but autoinducer 2 concentration, biofilm formation, and swarm motility decreased to 13.2, 11.8, and 50%, respectively. Survival at 5 days of nematodes (*Caenorhabditis elegans*) that were fed the pathogen without and with EGCG were 47.1 and 76%, respectively. Real-time PCR data indicated decreased transcriptional level in many quorum sensing–regulated virulence genes at 25 μg/ml. Our results suggest that EGCG at concentrations below its MIC has significant antipathogenic effects against *E. coli* O157:H7.

Bacteria use quorum sensing (QS) to regulate certain forms of gene expression by sensing their population density via hormone-like compounds, referred to as autoinducers (AI), that are excreted into the environment *(16). Escherichia coli* O157:H7, a foodborne pathogen, causes hemorrhagic colitis and hemolytic uremic syndrome. *E. coli* O157:H7 modulates the expression of its virulence genes, including those associated with Shiga toxin (Stx), siderophore synthesis, motility, and biofilm formation, via the AI-2 signaling pathway for interspecies communication *(27–29).* In patients with *E. coli* O157:H7 infection, antibiotic use is generally limited because the bacterial cells lysed by antibiotics are inclined to release an excessive quantity of Stx at the same time, thereby aggravating the patient's state and resulting in hemolytic uremic syndrome *(32).* To risk associated with antibiotic treatment, an antipathogenic treatment that attenuates virulence without bactericidal or bacteriostatic activity has been proposed, and QS has became a popular target in the development of antipathogenic drugs *(21).*

Epigallocatechin gallate (EGCG) is the most abundant polyphenolic antioxidant metabolite (catechin) in green tea. EGCG exerts bactericidal effects as well as antitoxic effects *(1, 18, 24).* Green tea extract has a wide spectrum of activity against 30 different pathogenic bacteria, including strains of *E. coli (7, 33, 34).* A methanol extract of green tea leaves protected Swiss white mice against mortality from *Salmonella* Typhimurium infection *(2, 7).* The extract also is active in vitro against many gram-positive and gram-negative bacteria. The average MICs of EGCG against *Staphylococcus aureus* (58 ± 16 μg/ml) were much lower than those against *Salmonella* Typhimurium (496 ± 15 μg/ml) and *E. coli* O157:H7 (539 ± 22 μg/ml) *(7, 25).* At subinhibitory concentrations, EGCG had a synergistic effect with antibiotics against antibiotic-resistant bacteria *(11, 23, 33, 36).* Although in numerous studies the synergistic effects of EGCG with a variety of antimicrobial compounds at concentrations below the MIC have been found *(11, 23, 36),* there have been relatively few reports describing the individual effect on bacteria of EGCG in this concentration range. At subinhibitory concentrations, EGCG inhibited biofilm formation in ocular staphylococcal isolates *(4).* EGCG also inhibited the extracellular release of Stx from *E. coli* O157:H7 *(30).* Recently, EGCG inhibited biofilm formation and swarm motility in *Pseudomonas putida* and *Burkholderia cepacia* by interfering with the AI-1 signaling system for intraspecies communication *(12).* However, neither the antipathogenic activities of EGCG in vivo nor the molecular basis underlying QS inhibition by EGCG were presented in that study.

In this study, we evaluated the potential of EGCG as an antipathogenic agent by investigating the relative changes in *E. coli* O157:H7 extracellular AI-2 activity, biofilm formation, swarm motility, and infection capability in the nematode *Caenorhabditis elegans,* a model host, in the presence of EGCG below inhibitory concentrations (5 to 100 μg/ml). The temporal response of expression in QS-regulated virulence genes also was investigated via real-time PCR. Our results indicate that EGCG can attenuate the virulence properties of *E. coli* O157:H7, particularly via the

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inhibition of motility and type 3 secretion systems, through its interference with AI-2 signaling. To the best of our knowledge, this is the first report of the effects of EGCG on QS-regulated virulence genes and corresponding virulence phenotypes in *E. coli* O157:H7.

MATERIALS AND METHODS

EGCG. High-performance liquid chromatography grade EGCG was purchased from Sigma (St. Louis, MO).

Bacterial strains, *C. elegans* strain, and culture conditions. *E. coli* O157:H7 ATCC 43894 (American Type Culture Collection, Manassas, VA), which produces Shiga toxins I and II, and its *luxS* mutant were grown at 30°C in Luria-Bertani (LB) medium. *Vibrio harveyi* BB170 was grown at 30°C on an orbital shaker (HB-201SF, Hanbaek Scientific Technology, Bucheon City, South Korea) at 175 rpm in AB medium *(9),* which consists of 0.3 M NaCl, 50 mM $MgSO_4$, 0.2% (wt/vol) casamino acids, 10 mM potassium phosphate buffer (pH 7.0), 1 mM L-arginine, 2% (wt/vol) glycerol, 1 mg/liter thiamine, and 10 μg/liter riboflavin. *C. elegans* wild-type strain N2 was obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, St. Paul). The nematodes were maintained at 16°C on nematode growth medium (NGM) plates previously seeded with *E. coli* OP50 as a food source and were manipulated via established techniques *(10).*

AI-2 activity assays. The bacterial supernatants were assayed using the techniques developed by Surette and Bassler *(31). E. coli* O157:H7 was grown overnight at 30°C in LB medium containing 0.5% (wt/vol) glucose, diluted 1:100 in the same fresh medium containing 5 to 100 μg/ml EGCG, and then grown at 30°C. Aliquots were collected at 4 h and utilized for both optical density measurements at 600 nm (OD_{600}) and the preparation of cell-free culture fluids. The supernatant was passed through a 0.2-μm-pore-size cellulose nitrate membrane filter (Whatman, Maidstone, UK). The reporter strain, *V. harveyi* BB170, was grown overnight in AB medium and then diluted 1:5,000 into fresh AB medium, and the cell-free supernatant from the *E. coli* O157:H7 was added to the diluted AB medium at a concentration of 10% (vol/vol). The luminescence values were measured with a luminescence counter (Wallac model 1450, PerkinElmer, Waltham, MA). Each experiment was conducted in triplicate.

Biofilm assay on microtiter plate. The crystal violet biofilm assay was adapted for this study *(19). E. coli* O157:H7 was grown overnight at 37°C in M9 medium supplemented with 0.4% glucose (wt/vol) and 0.4% casamino acids (wt/vol). This culture was diluted 1:100 in the same medium containing 5 and 50 μg/ml EGCG. The mixture was then transferred to polystyrene 96-well plates and incubated for 48 h at 25°C without shaking. To quantify the biofilm mass, the cell suspension was removed, and the plates were washed three times in water. The biofilm was then stained for 20 min with 0.1% crystal violet (wt/vol), and the extra dye was thoroughly removed with water. The remaining dye staining the biofilm was dissolved in 200 μl of 95% ethanol (vol/vol), and the OD_{595} was measured to quantify the biofilm mass. Each data bar represents the mean of six replicate wells, and the standard deviations were calculated.

Swarm motility assay. Tryptone broth (10 g/liter tryptone, 5 g/liter NaCl) containing 0.3% (wt/vol) agar *(8)* was used for the motility assays. Cultures of *E. coli* O157:H7 were grown overnight in liquid tryptone broth, diluted 100-fold in the same fresh medium, and grown to midexponential phase. Culture aliquots of 20 μl were mixed into the same volume of EGCG solution, and the mixtures were adjusted to 5, 25, and 100 μg/ml EGCG. Each tryptone plate was inoculated at the center with 5 μl of each mixture, and the plate was incubated for 11 h at 30°C and 67% relative humidity.

TABLE 1. *Oligonucleotides used for RT-PCR in this study*

Gene	Sequence of PCR primers (5′ to 3′)
qseA	Forward: GAGCGTTTCGTCCATCAGTC
	Reverse: TGCTCATGAACATCCTGCAC
qseB	Forward: GTGAGCCGGTACTGATCCTG
	Reverse: GTCGAGCATGACGTTACCGT
qseC	Forward: GTTCGACACCCAACTGATGC
	Reverse: CTCCGTTATCGCCATCGTTA
eae	Forward: ACCTCTTGTTGCTGCAGGTG
	Reverse: TAATCGCCGTTCAGAGATCG
escN	Forward: CAGTCAACGTTTAGCCGAGG
	Reverse: CTCCATTGGTCTGCCTATGC
espA	Forward: GCGAGTTCTTCGACATCGAC
	Reverse: TGGTTGACGCTTTAGATGCC
sepZ	Forward: CCTTCTGGTGCAGTAATGCC
	Reverse: TAGAGCTGCCAGAGCCGTAG
tir	Forward: TTACCTTCACAAACCGACGG
	Reverse: CGCATCGGATTTACAGGAAG
flhD	Forward: TGCACAGCGTCTGATTGTTC
	Reverse: GGGAATCTTGCGTCAACAGA
stx_1A	Forward: ATAGATCCAGAGGAAGGGCG
	Reverse: CTGCAACACGCTGTAACGTG
stx_2A	Forward: CTTCGGTATCCTATTCCCGG
	Reverse: GCCCTCGTATATCCACAGCA
rrsD	Forward: ATACCGCATAACGTCGCAAG
	Reverse: ATATTCCCCACTGCTGCCTC

RNA isolation. To obtain RNA for identification of the genes affected by EGCG, *E. coli* O157:H7 was grown overnight in LB medium supplemented with 0.5% (wt/vol) glucose, diluted 1:100 in the same medium containing 25 μg/l EGCG, and grown for 4 h at 30°C. The cells were harvested by centrifugation for 5 min at 4°C and 8,000 × *g* (VS-550 centrifuge, Vision Scientific Co., Buchon City, South Korea). The total RNA was isolated using an RNeasy Lipid Tissue Mini Kit (Qiagen Inc., Valencia, CA) in accordance with the manufacturer's protocols.

Real-time quantitative RT-PCR for gene transcription analyses. A Bioanalyzer 2100 (Agilent, Palo Alto, CA) was employed to confirm the purity and quantity of RNA in the samples. A two-step real-time quantitative reverse transcription PCR (qRT-PCR) was conducted with the RNA samples. The cDNA was prepared from 1 μg of total RNA using SuperScript III First-strand Synthesis Supermix for the qRT-PCR kit (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's recommendations. For the real-time qPCR, the synthesized cDNA was amplified with SYBR GreenER Universal kits (Invitrogen). The specific primers used in the real-time assays were designed with the GENETYX software package (Software Development Co., Tokyo, Japan) (Table 1). The qPCR amplification and detection were conducted on a Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). To analyze and quantify the gene expression data, Sequence Detection System software (SDS version 2.1, Applied Biosystems) was utilized. For standard curve plotting, a gel-purified standard DNA template representing each of the targets and the reference genes was diluted in a series from 10^{10} to 10^3 copies and employed for real-time qPCR, as described above. The

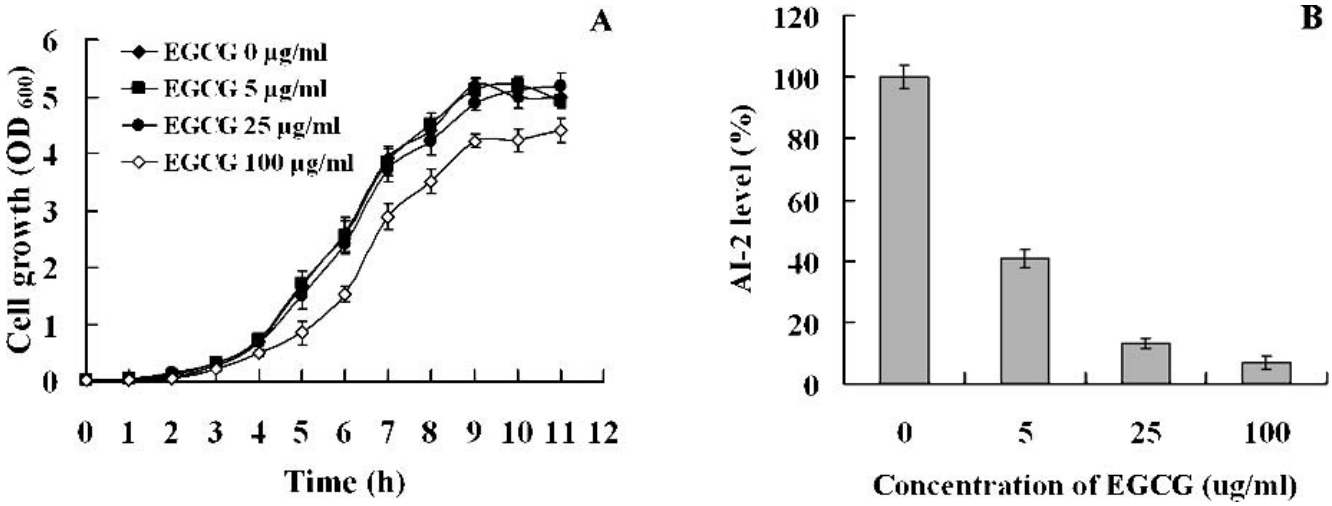

FIGURE 1. *Effect of EGCG on growth (A) and AI-2 production (B) in* E. coli *O157:H7.* E. coli *O157:H7 was grown in LB broth containing 0.5% (wt/vol) glucose with various concentrations of EGCG (solid diamonds, 0 μg/ml; solid squares, 5 μg/ml; solid circles, 25 μg/ml; open diamonds, 100 μg/ml) at 30°C with shaking. During culture, the OD_{600} was measured at the points indicated. AI-2 production was detected via a* V. harveyi *AI-2 bioassay using culture supernatants at 4 h. AI-2 level was expressed as a relative value based on the AI-2 value of the supernatant from culture of* E. coli *O157:H7 without EGCG. Three independent experiments were conducted, and the representative data are shown. The standard deviations are indicated by error bars.*

amplification efficiency for each target (*qseA, qseB, qseC, eae, escN, espA, sepZ, tir, flhD, stx_1B,* and *stx_2B*) and reference (*rrsD*) gene was derived from a standard curve plotted as the cycle threshold (C_T) versus the copies. The results were expressed as ratios of *qseA, qseB, qseC, eae, escN, espA, sepZ, tir, flhD, stx_1B,* and *stx_2B* mRNA to *rrsD* mRNA.

Growth of *E. coli* O157:H7 treated with various concentrations of EGCG on NGM plates. *E. coli* O157:H7 was grown overnight in LB broth at 30°C on an orbital shaker (HB-201SF, Hanbaek Scientific Technology) at 175 rpm. The serial dilutions of cultures were mixed with the same volume of EGCG solution, the mixtures were adjusted to 5, 25, and 100 μg/ml EGCG, and 20 μl of each mixture was dropped onto an NGM agar plate and incubated at 25°C.

***C. elegans* killing assays.** *C. elegans* killing assays were conducted as described previously but with modifications *(10).* All *E. coli* strains were grown overnight in LB broth at 37°C with aeration. Culture aliquots of 20 μl were mixed with the same volume of EGCG solution, and the mixture was adjusted to 5, 25, and 100 μg/ml EGCG and then spread onto NGM agar plates *(10).* The plates were dried at 25°C and then immediately utilized for the assays. Twenty nematodes (N2 strain) previously synchronized at the L4 stage were transferred to each plate, incubated for 24 h at 25°C, and transferred to a new plate every 24 h to allow for the determination of the survival rates of the original worms without the presence of progeny. A worm was considered dead when it did not respond to being touched by a platinum wire pick. Each assay was conducted in triplicate and was repeated three times.

RESULTS AND DISCUSSION

In a previous study, EGCG at subinhibitory concentrations inhibited AI-1 signaling, swarm motility, and biofilm formation in opportunistic bacterial pathogens, including *P. putida* and *B. cepacia (12).* These three factors have been recognized as contributing to pathogenesis in a variety of pathogenic bacteria. In the present study, we assessed the relative changes in *E. coli* O157:H7 extracellular AI-2 activity, biofilm formation, swarm motility, and infection capability in the nematode *C. elegans,* a model host, in the presence of EGCG at below inhibitory concentrations (5 to 100 μg/ml). To corroborate the phenotypic changes in *E. coli* O157:H7 in the presence of EGCG, a real-time PCR assay was used to evaluate the changes in the expression levels of QS-regulated genes.

Decreased extracellular AI-2 concentration in culture supernatant previously grown with EGCG at subinhibitory concentrations. In previous studies, when supernatants of bacterial cultures containing a QS inhibitor such as (5*Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone (furanone) were added to a AI-2 reporter strain *V. harveyi* BB170 (*luxN*::Tn*5*), the decreased levels of AI-2 were detected by the strain *(22, 29).* Using this method, the inhibitory effect of EGCG on AI-2 production of *E. coli* O157:H7 was determined in the present study. When *E. coli* O157:H7 was grown in LB medium supplemented with 100 μg/ml EGCG, bacterial growth was only slightly inhibited but the extracellular AI-2 concentration was significantly reduced to 6.9% (Fig. 1) in comparison with the AI-2 concentration in control cultures (0 μg/ml EGCG). However, at 5 and 25 μg/ml EGCG the AI-2 concentration was reduced to 40.6 and 13.2% without affecting the growth rate in a concentration-dependent manner (Fig. 1). These results indicate that EGCG inhibits QS in *E. coli* O157:H7. To determine whether EGCG is an AI-2–like molecule, the AI-2 concentration in the supernatant of the *ΔluxS* strain, which is unable to generate AI-2 synthase, was compared with that in the supernatant of the same strain grown with EGCG at all concentrations. With the addition of EGCG, the concentration of AI-2 did not change in the *ΔluxS* strain (data not shown), thereby indicating that EGCG is not an AI-2–like molecule.

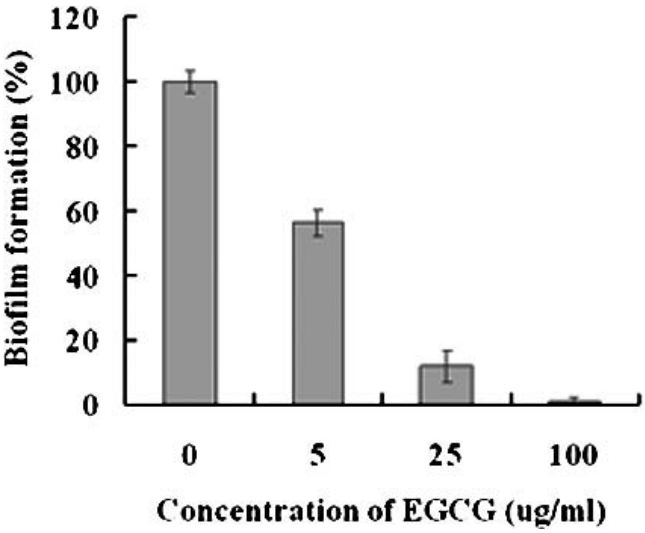

FIGURE 2. *Effect of EGCG on biofilm formation of* E. coli *O157:H7.* E. coli *O157:H7 was grown for 48 h in M9 media containing 5 to 100 μg/ml EGCG in polystyrene 96-well plates at 25°C without shaking. The biomass was measured after 48 h. Biofilm formation was expressed as relative values compared with values of cultures without EGCG. Each data bar is the mean of six replicate wells, and the standard deviations are indicated by error bars.*

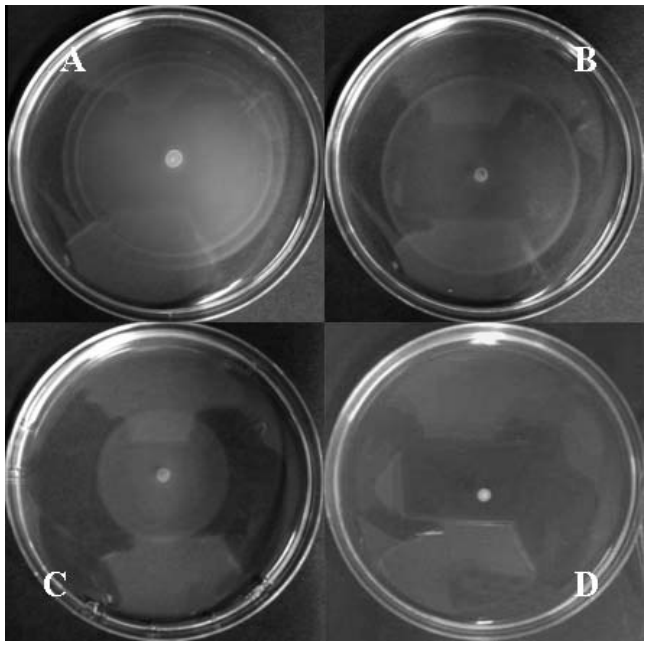

FIGURE 3. *Effect of EGCG on the motility of* E. coli *O157:H7.* E. coli *O157:H7 cultures (20 μl) were mixed with the same volume of EGCG solution, and the EGCG concentrations in the mixtures were adjusted: A, 0 μg/ml; B, 5 μg/ml; C, 25 μg/ml; D, 100 μg/ml. Cultures were inoculated with 5 μl of the mixture at the center of each tryptone plate, and the plates were incubated for 11 h at 30°C in a humid environment.*

Inhibition of biofilm formation and swarm motility. In a previous study *(21),* both swarming motility and biofilm formation of *E. coli* were inhibited in the presence of a QS inhibitor in a concentration-dependent manner. These results were supported by the decreased transcription levels in the genes related to biofilm formation and swarming motility via AI-2 as determined in a microarray experiment *(21).* In the present study, the swarm motility and biofilm assays were performed to determine whether EGCG inhibits the bacterial properties related to AI-2 signaling. To further clarify the antipathogenic activity of EGCG against *E. coli* O157:H7, the effect of EGCG on biofilm formation, one of the QS-regulated phenotypes *(8),* was evaluated. At 5 and 25 μg/ml EGCG, the biofilm formation of *E. coli* O157:H7 decreased to 56.4 and 11.8%, respectively, when compared with that in cultures without EGCG without affecting the growth of *E. coli* O157:H7 (Fig. 2). In addition to biofilm formation, at all tested concentrations (5, 25, and 100 μg/ml EGCG), swarming motility decreased 82.3, 50, and 0% in a concentration-dependent manner when compared with that in cultures without EGCG (Fig. 3). Previous studies have generated data indicating that pathogenicity is regulated by AI-2 in many bacterial pathogens, including *E. coli* O157:H7. Gonzalez-Barrios et al. *(8)* found that synthesized AI-2 directly stimulated biofilm formation and motility in strains of *E. coli.* Other researchers reported that biofilm formation in *Streptococcus mutans* was markedly attenuated by mutation on the *luxS* gene as compared with biofilms formed by the wild type *(35).* Similarly, Sperandio et al. *(28)* reported that the *E. coli* O157:H7 *luxS* mutant had reduced swarm motility and biofilm formation. Our data also suggest that the inhibitory effects of EGCG on biofilm formation and swarming motility are associated with reduced production of AI-2.

Increased survival rate of *C. elegans* fed *E. coli* O157:H7 in the presence of EGCG. *C. elegans* is a simple and economic invertebrate animal model that is being used increasingly for the study of mechanisms of microbial pathogenesis *(17, 26). C. elegans* was reported to be a good model to evaluate the virulence of *E. coli* O157:H7 and the antibacterial effectiveness of many types of chemical compounds *(5, 15). C. elegans* fed a nonpathogenic *E. coli* strain (OP50) lived longer than *C. elegans* fed *E. coli* O157:H7. In this study, *C. elegans* killing assays were conducted to determine whether EGCG could attenuate the virulence of *E. coli* O157:H7, thus protecting *C. elegans* fed on this microorganism from *E. coli* O157:H7 infection. The growth of *E. coli* O157:H7 on NGM plates was not affected in the presence of EGCG at concentrations of 5 to 100 μg/ml (Fig. 4). There were no significant differences in the survival rates of *C. elegans* for 4 days, but at 5 days the survival rate for the nematodes fed only *E. coli* O157:H7 were significantly lower than those for nematodes fed the pathogen in the presence of EGCG (Fig. 5). The attenuation of bacterial virulence by EGCG was dependent on its concentration. Survival rates of the nematodes for 5 days were 64, 76, and 86% fed *E. coli* O157:H7 cultures with 5, 25, and 100 μg/ml EGCG, respectively. Rasmussen et al. *(20)* found that a QS mutant of *Pseudomonas aeruginosa* killed fewer nematodes than did the wild-type strain. Kim et al. *(14)* also found that *E. coli* O157:H7 in the presence of exogenous AI-2 molecules killed more nematodes than it did in the absence of the AI-2 molecules. Brown et al. *(6)* reported that the life span of *C. elegans* fed a nonpatho-

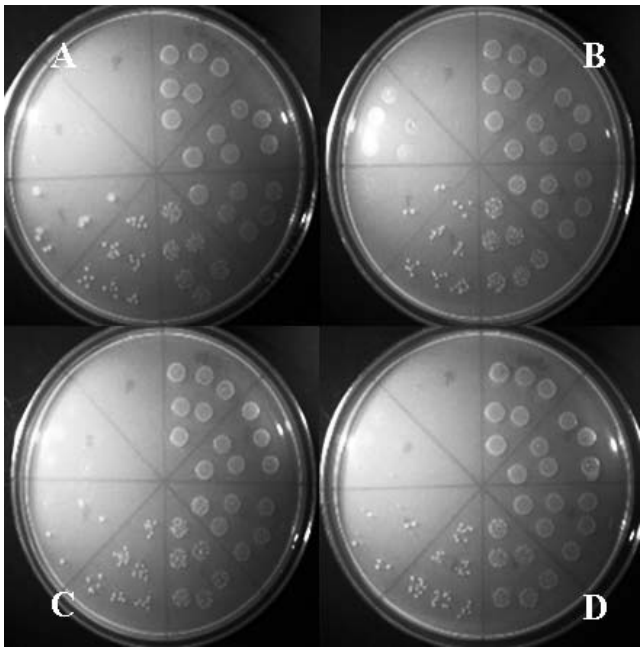

FIGURE 4. *Effect of EGCG on the growth of* E. coli *O157:H7 on NGM plates.* E. coli *O157:H7 was grown overnight in LB broth at 37°C with aeration. Serial dilutions of the cultures were mixed with the same volume of EGCG solution, and the EGCG concentrations in the mixtures were adjusted: A, 0 μg/ml; B, 5 μg/ml; C, 25 μg/ml; D, 100 μg/ml. NGM agar plates were inoculated with 20 μl of the mixture and incubated at 25°C. The number of colonies was recorded after 48 h.*

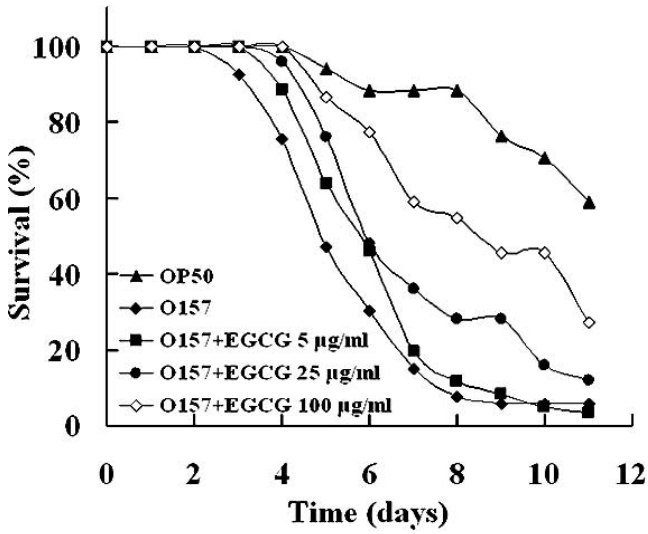

FIGURE 5. *Effects of EGCG on the lifespan of* C. elegans*. Wild-type N2 nematodes were placed on plates spread with a mixture containing cultures of* E. coli *O157:H7 and EGCG. The final EGCG concentration of the mixtures was adjusted: solid diamonds, 0 μg/ml; solid squares, 5 μg/ml; solid circles, 25 μg/ml; open diamonds, 100 μg/ml. The nematodes were transferred daily to fresh plates. The survival rates were scored daily and expressed as percentages. Three independent experiments were conducted, and the representative data are shown.*

genic *E. coli* OP50 with EGCG was similar to that of *C. elegans* fed only *E. coli* OP50. The results of our *C. elegans* killing assay suggest that EGCG can protect the nematodes against pathogenic attack by inhibiting QS.

EGCG reduces the transcription of major virulence genes in *E. coli* O157:H7. To determine whether reduced AI-2 activity induced by EGCG also can reduce the expression of virulence genes regulated by QS, RT-PCR assays were conducted. The transcription levels of the *eae, escN, espA, sepZ,* and *tir* genes in the locus for enterocyte effacement (LEE)–encoded type III secretion system (TTSS) decreased to 72.1, 87.3, 79.5, 78.8, and 90.8%, respectively, when grown with 25 μg/ml EGCG (Fig. 6). The reduced transcription levels were correlated with the reduction in the transcription level of *qseA,* the activator of the LEE-encoded virulence genes (Fig. 6). *E. coli* O157:H7 induces a characteristic histopathology in intestinal cells, known as attaching and effacing (A/E). The A/E phenotypes are encoded by the LEE pathogenicity island *(13).* The LEE pathogenicity island harbors (i) the *sep* and *esc* genes encoding for a TTSS, (ii) the *eae* gene for the adhesin intimin, (iii) the *espABD* genes for proteins secreted by the TTSS, and (iv) the *tir* gene for the translocated intimin receptor. These genes in the LEE island are activated by the transcription of the *qseA* gene, which is activated by QS via AI-2 molecules *(27, 29).*

The transcription levels in the *qseB* and *qseC* genes also were decreased to 40.5 and 40.5% in the presence of EGCG (Fig. 6). This reduced transcription level appeared to affect the transcription of *flhD,* which was reduced to 48.6%. QS also activates the expression of genes involved in the assembly of flagella and motility via the activation of *flhDC* transcription *(29).* In a previous study, experiments revealed that *E. coli* O157:H7 required flagella for the persistent colonization of chickens *(3).*

Transcription of the genes associated with production of Shiga toxin (stx_1B and stx_2B) decreased to 38.8 and 58.1% in the presence of EGCG (Fig. 6). Other researchers reported that 50 to 200 μg/ml EGCG gradually inhibited the extracellular release of Stx from *E. coli* O157:H7 *(30).* Sperandio et al. *(28)* also observed a threefold reduction in *stx* transcription in the *luxS* mutant compared with the *stx* transcription in the wild-type strain. Our data indicate that EGCG can reduce Stx production.

Collectively, the results of our RT-PCR assays demonstrate that transcription of the genes encoding for major QS-regulated virulence factors was reduced in the presence of EGCG, thereby suggesting that EGCG interferes with AI-2 signaling.

In this study, EGCG was capable of interfering with QS in enterohemorrhagic *E. coli* O157:H7 by decreasing AI-2 production. In the presence of EGCG, extracellular AI-2 concentration, exopolysaccharide production, swarm motility, and virulence in *E. coli* O157:H7 were reduced. These phenotypic changes in *E. coli* O157:H7 in the presence of EGCG were supported by the results of our RT-PCR assay. Our results indicate that EGCG is a QS inhibitor that can be utilized as an antipathogenic agent for the prevention of *E. coli* O157:H7 infection and that EGCG also may attenuate virulence in other QS pathogens. In cases of *E. coli* O157:H7 infection, EGCG could be utilized

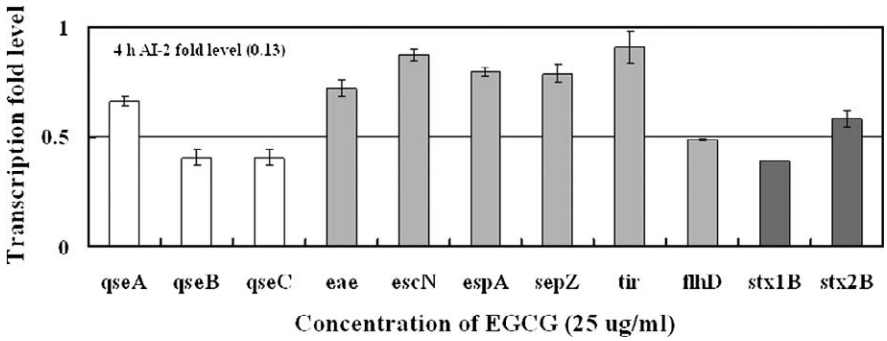

FIGURE 6. *Real-time RT-PCR assay of* qseA, qseB, qseC, eae, escN, espA, sepZ, tir, flhD, stx_1B, *and* stx_2B *from cultures of* E. coli *O157:H7 incubated in LB broth containing 0.5% (wt/vol) glucose with and without 25 μg/ml EGCG. The sampling time was 4 h. The mRNA levels of each gene were adjusted to that of* rrsD *mRNA, which was used as the internal standard. The error bars indicate the standard deviations from the means. The values in parentheses are relative folds for the culture with 25 μg/ml EGCG compared with the AI-2 value of the culture supernatant without EGCG.*

in an alternative treatment regimen for those patients whose symptoms could be aggravated by antibiotic use.

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