Stationary-Phase Quorum-Sensing Signals Affect Autoinducer-2 and Gene Expression in *Escherichia coli*

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Quorum sensing via autoinducer-2 (AI-2) has been identified in different strains, including those from *Escherichia*, *Vibrio*, *Streptococcus*, and *Bacillus* species, and previous studies have suggested the existence of additional quorum-sensing signals working in the stationary phase of *Escherichia coli* cultures. To investigate the presence and global effect of these possible quorum-sensing signals other than AI-2, DNA microarrays were used to study the effect of stationary-phase signals on the gene expression of early exponential-phase cells of the AI-2-deficient strain *E. coli* DH5α. For statistically significant differential gene expression ($P < 0.05$), 14 genes were induced by supernatants from a stationary culture and 6 genes were repressed, suggesting the involvement of indole (induction of *tnaA* and *tnaL*) and phosphate (repression of *phoA*, *phoB*, and *phoU*). To study the stability of the signals, the stationary-phase supernatant was autoclaved and was used to study its effect on *E. coli* gene expression. Three genes were induced by autoclaved stationary-phase supernatant, and 34 genes were repressed. In total, three genes (*ompC*, *ptsA*, and *btuB*) were induced and five genes (*nupC*, *phoB*, *phoU*, *argT*, and *ompF*) were repressed by both fresh and autoclaved stationary-phase supernatants. Furthermore, supernatant from *E. coli* DH5α stationary culture was found to repress *E. coli* K-12 AI-2 concentrations by 4.8-fold ± 0.4-fold, suggesting that an additional quorum-sensing system in *E. coli* exists and that gene expression is controlled as a network with different signals working at different growth stages.

Quorum sensing, the regulation of gene expression by producing and responding to secreted autoinducers (AIs) whose concentrations reflect the population density (2), commonly exists in bacteria. Gram-negative bacteria use acylated homoserine lactones as AIs, and gram-positive bacteria use oligopeptides (2, 16). When the cell density is high, the binding of AIs to cell receptors regulates gene expression for a variety of phenotypes, such as production of virulence factors (4), protein production (7), siderophore synthesis (29), bioluminescence (5), biofilm formation (6), and plasmid conjugation (15). Generally, each bacterial species uses its own signal; however, a common AI-2 signal has been discovered for interspecies communication (30, 31, 37). *Escherichia coli* does not produce acylated homoserine lactone, but it possesses the AI-2 quorum-sensing system (30). Recent studies have found that *E. coli* O157:H7 uses AI-2 to control the expression of virulence factors, type III secretion, chemotaxis, flagellar synthesis, and motility (24–26) and that *E. coli* K-12 uses AI-2 to control chemotaxis, motility, and flagellar synthesis (D. Ren, A. Bedzyk, S. M. Thomas, R. W. Ye, and T. K. Wood, submitted for publication). In addition, *E. coli* RP37 uses AI-2 to control cell aggregation (17). In *Salmonella enterica* serovar Typhimurium, AI-2 concentrations are maximal in mid-exponential-phase growth, and it is degraded in the stationary phase

by an unknown mechanism (30). *E. coli* DH5α does not produce AI-2 due to a 60-amino-acid deletion stemming from a 1-bp deletion that results in early truncation of *luxS* (formerly *ygaG*) (31).

Several lines of evidence suggest the existence of additional *E. coli* quorum-sensing signals besides AI-2. Previously, Withers and Nordström (36) reported that *E. coli* produces an extracellular factor in late exponential phase to early stationary phase that represses chromosomal replication. By using the bicistronic reporter transposon mini-Tn5 *lacZ-tet/1*, Baca-DeLancey et al. (1) found some *E. coli* genes (*cysK*, *astD*, *tnaB*, and *gabT*) that were activated by extracellular signals from stationary-phase, 0.5× Luria-Bertani (LB) medium cultures that are stable at pH 12 (20 min) and 100°C (10 min). These genes have functions for pyruvate and succinate production (1). Recently, indole, purified from stationary-phase *E. coli* 0.5× LB medium cultures, was found to induce the expression of *gabT*, *astD*, and *tnaB* (32). In addition, Sperandio et al. (27) found a new AI (AI-3) in *E. coli* O157:H7 involved in communication between the bacterium and its eukaryotic host.

With the ability to simultaneously quantify the expression of all genes in one organism whose genome has been sequenced (39), DNA microarrays have been successfully used to study bacterial responses to different stimuli such as heat shock and other stresses (12, 35, 40), quorum sensing (8, 26), anaerobic metabolism (38), sporulation (10), and biofilm formation (20, 23, 28, 34). To further study the roles of *E. coli* stationary-phase extracellular signals, DNA microarrays were used here to study *E. coli* DH5α (AI-2⁻) (30) gene expression with and without the addition of *E. coli*-conditioned medium. The effect of stationary-phase quorum-sensing signals on AI-2 synthesis

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in the exponential phase was also investigated by using *E. coli* K-12 (AI-2^{+}). This is the first study to investigate the effect of *E. coli* stationary-phase quorum-sensing signals on *E. coli* global gene expression by using DNA microarrays and to show that stationary-phase quorum-sensing signals repress AI-2 concentrations in the exponential phase.

MATERIALS AND METHODS

Bacterial strains and culture media. *E. coli* DH5α [*luxS supE44 ΔlacU169* (φ80 *lacZΔM15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*] (22, 31) was used to investigate the effect of stationary-phase signals on the gene expression of early exponential *luxS* mutant cells. *E. coli* K-12 (ATCC 25404) was used to study the effect of stationary-phase signals on AI-2 production. *Vibrio harveyi* BB170 (AI-1 sensor[−], AI-2 sensor[+]) was used as the reporter to measure AI-2 activity (21, 30). LB medium (22) containing 10 g of tryptone/liter, 5 g of yeast extract/liter, and 10 g of NaCl/liter with pH adjusted to 7.5 (1) was used to grow the *E. coli* cells. AI bioassay (AB) medium (11) was used to grow *V. harveyi* BB170, and LM medium (3) was used to determine the number of *V. harveyi* CFU.

Preparation of conditioned media. *E. coli* DH5α was grown in LB medium (pH 7.5) overnight and was diluted 1:100 in the same fresh medium. When the culture grew to an optical density at 600 nm (OD_{600}) (DU 640 spectrophotometer; Beckman, Fullerton, Calif.) of 1.65 (1.2×10^8 cells/ml), the cells were precipitated by centrifuging at $10,000 \times g$ for 10 min at 4°C and the supernatant was collected. Note that an OD of 0.2 indicated exponential growth of the *E. coli* cultures, an OD of 0.9 indicated transition from late exponential phase to early stationary phase, and an OD of 1.6 indicated stationary-phase growth. The harvested supernatant was split equally into two portions. One portion was supplemented with tryptone and yeast extract to 0.5 times of that of LB medium, and the pH was adjusted to 7.5. The resulting solution was sterilized by filtering through a 0.2-μm-pore-size filter and will be referred to as fresh conditioned medium (FCM). Another portion of the supernatant was autoclaved for 2 h (121°C, 15 lb/in[2]), tryptone and yeast extract were added, the pH was adjusted to 7.5, and the supernatant was filter sterilized; this medium is termed autoclaved conditioned medium (ACM). Sterile 0.5× LB medium (pH 7.5) was used as the negative control medium to investigate the effect of FCM and ACM on gene expression.

Growth in conditioned media and RNA isolation. To prepare cell cultures for RNA isolation, 200 μl of an overnight *E. coli* DH5α culture (grown in LB, pH 7.5) was added to 150 ml of the same fresh medium and was grown to an OD of 0.2 (1.4×10^7 cells/ml). Then the culture was split equally into three portions and was centrifuged at $10,000 \times g$ for 5 min at 4°C. The cell pellets were washed once with 40 ml of ice-cold 0.85% NaCl buffer and were centrifuged at $10,000 \times g$ for 5 min at 4°C. The cell pellets were resuspended in 40 ml each of 0.5× LB (pH 7.5), ACM, or FCM and were incubated with shaking for 30 min at 37°C. All three cultures grew to an OD of around 0.3 after incubation. Then the cells were centrifuged at $10,000 \times g$ for 3 min at −10°C, resuspended in 6 ml of ice-cold 0.85% NaCl buffer, split into four 1.5-ml portions, centrifuged at $10,000 \times g$ for 15 s at room temperature, and flash-frozen in a dry-ice–ethanol bath. The cells were kept at −80°C until RNA isolation.

To lyse the cells, 1.0 ml of RLT buffer (Qiagen, Inc., Valencia, Calif.) and 0.2 ml of 0.1-mm zirconia/silica beads (Biospec, Bartlesville, Okla.) were added to the frozen bead beater tubes containing the cell pellets. The tubes were closed tightly and were beat for 30 s at the maximum speed in a mini-bead beater (catalog no. 3110BX; Biospec). The total RNA was isolated by following the protocol of the RNeasy Mini Kit (Qiagen), including an on-column DNase digestion with RNase-free DNase I (Qiagen). An OD_{260} reading was used to quantify the RNA yield. OD_{260}/OD_{280} and 23S/16S rRNA readings were measured to check the purity and integrity of RNA (RNeasy Mini handbook; Qiagen).

DNA microarrays. The antisense *E. coli* DNA microarrays were prepared as described previously (33). In detail, each gene probe was synthesized by PCR and has a size of the full open reading frame of the corresponding gene (200 to 2,000 nt). The double-strand PCR products were denatured in 50% dimethyl sulfoxide and were spotted onto aminosilane slides (Full Moon Biosystems, Sunnyvale, Calif.) as probes to hybridize with the mRNA-derived cDNA samples. It has been shown that each array can detect 4,228 of the 4,290 *E. coli* open reading frames (33). Each gene has two spots per slide. Eight amplified *Klebsiella pneumoniae* genes and 12 cDNA clones from plants were spotted on each slide as negative controls, while there were 76 *E. coli* PCR products spotted on each slide as positive controls (33).

Synthesis of Cy3- or Cy5-labeled cDNA. To convert the total RNA into labeled cDNA from the *E. coli* DH5α samples treated with FCM, ACM, or 0.5× LB, reverse transcription was performed in 1.5-ml microcentrifuge tubes (Fisher) to which 6 μg of total RNA and 6 μg of random hexamer primers (Invitrogen Corp., Carlsbad, Calif.) were added. The volume was adjusted to 24 μl with RNase-free water (Invitrogen). The mixture was incubated for 10 min at 70°C, followed by 10 min at room temperature for annealing; then the reaction components were added, consisting of 8 μl of 5× SuperScript II reaction buffer (Invitrogen), 4 μl of 0.1 M dithiothreitol (Invitrogen), 1 μl of a deoxynucleoside triphosphate mix (a 2 mM concentration each of dATP, dGTP, and dTTP and 1 mM dCTP), 1 μl of 0.5 mM Cy3- or Cy5-labeled dCTP (Amersham Biosciences, Piscataway, N.J.), and 2 μl of SuperScript II reverse transcriptase (10 U/μl, Invitrogen). cDNA synthesis was conducted at 42°C for 2 h and was stopped by heating at 94°C for 5 min. After cDNA synthesis, the RNA template was removed with 2 μl of 2.5 M NaOH. The pH was neutralized with 10 μl of 2 M HEPES buffer, and the cDNA was purified with a Qiaquick PCR Mini Kit (Qiagen). The efficiency of labeling was checked via absorbance at 260 nm for the cDNA concentration, at 550 nm for Cy3 incorporation, and at 650 nm for Cy5 incorporation.

Hybridization and washing. The cDNA samples of *E. coli* DH5α treated with FCM or 0.5× LB (6 μg of each) were each labeled with both Cy3 and Cy5 dyes to remove artifacts related to different labeling efficiencies; hence, each experiment required at least two slides. The Cy3-labeled FCM sample and Cy5-labeled 0.5× LB sample were hybridized on the first slide. Similarly, the Cy5-labeled FCM sample and Cy3-labeled 0.5× LB sample were hybridized on the second slide. Since each gene has two spots on a slide, the two hybridizations generated eight data points for each gene (four points for the FCM sample and four points for the 0.5× LB sample). DNA microarrays for the *E. coli* DH5α treated with ACM or 0.5× LB were performed in an analogous manner.

The DNA microarrays were incubated in prehybridization solution (3.5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate] [Invitrogen], 0.1% sodium dodecyl sulfate [SDS] [Invitrogen], and 0.1% bovine serum albumin [Invitrogen]) at 45°C for 20 min. The arrays were rinsed with double-distilled water and were spun dry by centrifugation. Labeled cDNA (6 μg) was concentrated to 10 μl of total volume and was mixed with 10 μl of 4× cDNA hybridization solution (Full Moon Biosystems) and 20 μl of formamide (EM Science, Gibbstown, N.J.). The hybridization mix was heated to 95°C for 2 min and was added to the DNA microarrays; each array was covered with a coverslip (Corning, Big Flats, N.Y.) and was incubated overnight at 37°C for hybridization. When the hybridization was finished, the coverslips were removed in 1× SSC–0.1% SDS at room temperature, and the arrays were washed once for 5 min in 1× SSC–0.1% SDS at 40°C, twice for 10 min in 0.1× SSC–0.1% SDS at 40°C, and twice for 1 min in 0.1× SSC at 40°C. The arrays were quickly rinsed by dipping in room-temperature double-distilled water and were then spun dry by centrifugation.

Image and data analysis. The hybridized slides were scanned with the Generation III Array Scanner (Molecular Dynamics Corp.). Readings at 570 and 670 nm was used to quantify the probes labeled with Cy3 and Cy5 separately. The signal was quantified with Array Vision 4.0 or 6.0 software (Imaging Research, St. Catherines, Ontario, Canada). Genes were identified as differentially expressed if the expression ratio was greater than 2.4-fold (for the data set of FCM versus 0.5× LB) or 1.9-fold (for the data set of ACM versus 0.5× LB) and if P (found by t test) was less than 0.05. P values were calculated on log-transformed, normalized intensities. Including the P criterion ensures the reliability of the induced- and/or repressed-gene list. Normalization was relative to the median total fluorescent intensity per slide per channel. The gene functions were obtained from the NCBI database (http://www.ncbi.nlm.nih.gov/).

RNA dot blotting. Digoxigenin-labeled DNA probes of six genes, *phoB*, *ompC*, *ptsA*, *btuB*, *nupC*, and *b1498*, were synthesized by using the PCR DIG Probe Synthesis Kit (Roche Applied Science, Mannheim, Germany). The PCR was performed in 30 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 40 s. The final extension was at 72°C for 7 min. The probes have lengths of 172 to 400 bp (see Table 5 for specific primers used). Total RNA (1.25, 2.5, or 5 μg) from independent cell cultures (different experiments from those used for the DNA microarrays but identical culture conditions) was blotted on positively charged nylon membranes (Boehringer Ingelheim, Ridgefield, Conn.) by using a Bio-Dot Microfiltration Apparatus (Bio-Rad, Richmond, Calif.). Total RNA was fixed by baking for 2 h at 80°C. DNA probes (about 400 ng; a serial dilution of RNA samples was tested to ensure excess of the DNA probes) were denatured in boiling water for 5 min before hybridizing to RNA. Hybridization (50°C, 16 h) and washing were according to the protocol for digoxigenin labeling and detection (Roche Applied Science). To detect the signal, disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-[5-chloro]tricyclo[3.3.1.1,7]decan}-4-yl) phenyl phosphate (Roche Applied Science) was used as a substrate to give chemilumi-

TABLE 1. Genes induced by the fresh *E. coli* stationary-phase supernatant[a]

Gene	b no.	Expression ratio	Description
Known functions			
ptsA	b3947	21.4	PEP-protein phosphotransferase system enzyme I
btuB	b3966	14.2	Vitamin B$_{12}$ receptor precursor
tnaL	b3707	10.6	Tryptophanase leader peptide, degradation of small molecules
ompC	b2215	4.1	Outer membrane protein c precursor
tnaA	b3708	3	Tryptophanase
cysA	b2422	2.6	Transport of small anionic molecules
fliK	b1943	2.6	Flagellar hook-length control protein
aldA	b1415	2.5	Carbon energy metabolism
Unknown functions			
b1498	b1498	3.9	Putative enzyme, not classified
b1285	b1285	3.8	ORF, unknown
evgA	b2369	3	Putative positive transcription regulator (sensor EvgS)
yqgD	b2941	2.8	ORF, unknown
b2845	b2845	2.6	Putative transporter protein
ybaJ	b0461	2.4	ORF, unknown

[a] ORF, open reading frame. Boldfaced designations indicate those genes induced by both FCM and ACM.

TABLE 2. Genes repressed by the fresh *E. coli* stationary-phase supernatant[a]

Gene	b no.	Expression ratio	Description
nupC	b2393	−5.3	Transport of small molecules: nucleosides, purines, pyrimidines
phoB	b0399	−4.5	Positive response regulator for *pho* regulon; sensor is PhoR (or CreC)
phoU	b3724	−4.4	Negative regulator for *pho* regulon and putative enzyme in phosphate metabolism
argT	b2310	−4.2	Transport of small molecules: amino acids and amines
ompF	b0929	−3.8	Membrane and outer membrane constituents
phoA	b0383	−2.7	Alkaline phosphatase

[a] Boldfaced designations indicate genes repressed by both FCM and ACM.

nescence, and the light was recorded by Biomax X-ray film (Kodak, Rochester, N.Y.).

Effect of stationary-phase signals on AI-2 concentrations in *E. coli* K-12 cultures. To study if the unknown signals from the stationary phase of *E. coli* culture may cause the degradation of AI-2, FCM was supplemented with 0.5% glucose and was used to grow *E. coli* K-12 from an OD of 0.02 to one of 0.9 in 2.25 h. As the control sample, sterile 0.5× LB medium was supplemented with 0.5% glucose and was used to grow *E. coli* K-12 to an OD of 0.9 under identical conditions. Then both samples were centrifuged at 10,000 × *g* for 10 min at 4°C to remove the cells, and the supernatants were filter sterilized and were kept at −20°C until the AI-2 assay.

AI-2 AI bioassay. *E. coli* supernatants were assayed using the method of Surette and Bassler (21, 30). The reporter strain *V. harveyi* BB170 was grown in AB medium overnight and was diluted 1:5,000 into the fresh AB medium; then the cell-free supernatants from the *E. coli* samples were added at a concentration of 10% (vol/vol). The time course of bioluminescence was measured with a 20/20 luminometer (Turner Design, Sunnyvale, Calif.) and was reported in relative light units. The cell density of the *V. harveyi* reporter strain was measured by spreading the cells on LM plates and by counting CFU after 24 h. The experiment was conducted in duplicate.

RESULTS

Comparison of gene expression profiles affected by FCM and ACM. To investigate the effect of stationary-phase signals on a genetic basis and to study the stability of the signals, DNA microarrays were used to obtain the gene expression profiles of *E. coli* treated with FCM, ACM, or 0.5× LB. Since the unknown signal studied by Baca-DeLancey et al. (1) was stable at 100°C for 10 min, the ACM was prepared to investigate the heat sensitivity of the signals in the present study. In addition, nutrients (tryptone and yeast extract) were added in FCM and ACM to ensure that the differential gene expression was not caused by starvation.

According to the microarray results, FCM significantly induced 14 genes (Table 1) and significantly repressed 6 genes (Table 2). Similarly, ACM induced 3 genes (Table 3) and

repressed 34 genes (Table 4). The gene expression patterns under these two sets of conditions agree well with each other. All three genes (*ompC*, *ptsA*, and *btuB*) induced by ACM were also induced by FCM. Similarly, five out of the six genes (*nupC*, *phoB*, *phoU*, *argT*, and *ompF*) repressed by FCM were also repressed by ACM. However, FCM induced more genes than ACM, while ACM repressed more genes than FCM. Hence, the signals may have been partially degraded by autoclaving.

Genes induced by stationary-phase signals. The genes induced by FCM have functions for energy metabolism (*aldA*), anion and carbohydrate transport (*cysA*, *ptsA*), flagellar synthesis (*fliK*), membrane protein (*ompC*), indole production (*tnaA*, *tnaL*), and unknown functions (*b1285*, *b1498*, *b2845*, *ybaJ*, and *yqgD*) (Table 1). The induction of *tnaA* agrees with the recent report that *E. coli* MG1655 produces indole at 340 μM in the stationary phase (32).

The induction of *ompC* by the stationary-phase signals is consistent with the fact that the two important outer membrane porin proteins, OmpC and OmpF, are subject to many environmental factors (18). *ompC* and *ompF* are controlled by OmpR-phosphate (OmpR-P) in an opposite manner. OmpR-P activates *ompF* at low osmolarity, while it represses *ompF* and activates *ompC* at high osmolarity (18). In addition, *ompF* was found to be repressed when cells enter the stationary phase (18), and the expression of *ompC* was found to increase in *E. coli* biofilms which contain a high density of cells (19). Therefore, the induction of *ompC* by stationary-phase signals suggests that the *E. coli* cells are encountering high osmolarity in the stationary phase.

Genes repressed by stationary-phase signals. As expected, *ompF* was repressed by stationary-phase signals. In addition,

TABLE 3. Genes induced by the autoclaved *E. coli* stationary-phase supernatant[a]

Gene	b no.	Expression ratio	Description
ptsA	b3947	3.9	PEP-protein phosphotransferase system enzyme I
btuB	b3966	3.0	Vitamin B$_{12}$ receptor precursor
ompC	b2215	2.5	Outer membrane protein c precursor

[a] Boldfaced designations indicate genes induced by both FCM and ACM.

TABLE 4. Genes repressed by the autoclaved *E. coli*
stationary-phase supernatant[a]

Gene	b no.	Expression ratio	Description
Known functions			
phoB	b0399	−35.7	Phosphate regulon regulatory protein PhoB
nupC	b2393	−8.8	Nucleoside permease (nucleoside-transport system protein)
phoU	b3724	−6.5	Phosphate transport system regulatory protein
phoR	b0400	−5.6	Phosphate regulon sensor protein PhoR
argT	b2310	−5	Lysine-arginine-ornithine-binding periplasmic protein precursor
nlpC	b1708	−4.5	Probable lipoprotein N1pC precursor
dsdX	b2365	−4.4	DsdX permease
gltB	b3212	−3.4	Glutamate synthetase, large subunit
ompF	b0929	−3.3	Outer membrane protein f precursor
guaC	b0104	−3.2	GMP reductase
phoE	b0241	−3.2	Outer membrane pore protein E
bcr	b2182	−3.1	Bicyclomycin resistance protein (sulfonamide resistance)
nupG	b2964	−3	Nucleoside-transporting protein NupG
carA	b0032	−2.7	Carbamoyl-phosphate synthetase glutamine chain
gltD	b3213	−2.6	Glutamate synthetase beta subunit
carB	b0033	−2.5	Glutamine-hydrolyzing
ldcC	b0186	−2.4	Lysine decarboxylase, constitutive
rfbX	b2037	−2.3	O-antigen transporter
sdiA	b1916	−2.3	SdiA regulatory protein
grxA	b0849	−2.1	Glutaredoxin 1
phoH	b1020	−2.1	PhoH protein (phosphate starvation-inducible protein PsiH)
aroP	b0112	−2	Aromatic amino acid transport protein AroP
flgJ	b1081	−2	Flagellar basal body protein FlgJ
pyrE	b3642	−2	Orotate phosphoribosyltransferase
Unknown functions			
yciH	b1282	−5.1	Hypothetical protein
b2063	b2063	−4.9	Putative transport, not classified
b2639	b2639	−3.9	Unknown
b1017	b1017	−3.5	Unknown
b1018	b1018	−3.1	Unknown
b1520	b1520	−3	Open reading frame, unknown
ycdB	b1019	−2.9	Hypothetical protein
yffG	b2468	−2.6	Putative enzyme, not classified
yafL	b0227	−2.5	Hypothetical protein
ybgC	b0736	−2.2	Hypothetical protein

[a] Boldfaced designations indicate genes repressed by both FCM and ACM.

the *pho* operon was also repressed by stationary-phase signals (Table 2). While five genes were consistently repressed by FCM and ACM, more genes (34 genes) were repressed by ACM. The increase in number of repressed genes may be caused by the heat treatment of the conditioned medium, which may degrade chemicals and produce toxic products. The high temperature may lyse chemicals such as peptides and amino acids. In addition, the high temperature and pressure may facilitate a variety of reactions between reactive groups (such as amines), which may lead to the production of unfavorable compounds for the cells.

Validation of the DNA microarray results with RNA dot blotting. To confirm the gene expression profiles obtained from the DNA microarray analysis, total RNA was isolated from independent samples and was prepared in the same manner as the DNA microarray experiments, and the expression levels of six representative genes (*phoB*, *ompC*, *ptsA*, *btuB*, *nupC*, and *b1498*) were confirmed with RNA dot blotting (Table 5). For example, *phoB*

was repressed fivefold by FCM in the DNA microarray results and was also repressed fivefold by FCM in the RNA dot blotting experiment. Hence, the DNA microarray results provided valid information about the differential gene expression profiles due to the addition of FCM or ACM.

Stationary-phase signals repress the AI-2 concentration in *E. coli* K-12 exponential cultures. Since AI-2 is degraded when *S. enterica* serovar Typhimurium cells enter the stationary phase (30), it was hypothesized that the addition of stationary-phase signals to an *E. coli* culture would repress the AI-2 concentration. To test this hypothesis, FCM and 0.5× LB were each supplemented with 0.5% glucose and were used to grow *E. coli* K-12 from an OD of 0.02 to an OD of 0.9; glucose was added to produce AI-2 (30). The supernatants were then used for the AI-2 bioassay.

It was found that the stationary-phase signals repressed by 4.8-fold ± 0.4-fold the AI-2 concentration in FCM (79% repressed), compared to the concentration of AI-2 found in 0.5× LB (Fig. 1). This agrees well with a previous study that indicated that 75% of AI-2 was degraded when *S. enterica* serovar Typhimurium LT2 was grown for 20 h (14 h after the AI-2 reached the maximum) (30).

DISCUSSION

Although the actual compounds need to be identified, this study indicates that *E. coli* has stationary-phase signals that specifically induce or repress certain genes to adjust to high cell density. Since the effects of FCM and ACM were compared with results for 0.5× LB (both FCM and ACM contain 0.5× LB), the induction or repression of genes was not due to the absence of needed nutrient components. It is well accepted that bacteria use quorum sensing to discern their population and adjust gene expression to take advantage of the resources and attack eukaryotic hosts (2). Given that *E. coli* AI-2 controls different phenotypes such as flagellar synthesis, motility, and virulence expression (25, 26), an understanding of the mechanism of AI-2 degradation is necessary. In this study, signals from stationary-phase cultures were found to decrease AI-2 concentrations significantly in *E. coli* K-12 cultures. Hence, there may be different quorum-sensing signals working at different growth stages enabling bacteria to discern their growth state. The genes induced by these stationary-phase signals are candidates for further studies, since it is possible that some of them encode factors that degrade AI-2 or induce enzymes that degrade AI-2. It is also possible that the stationary-phase signals repress AI-2 synthesis (via *luxS*) and allow basal AI-2 degradation to reduce AI-2 concentrations in the stationary phase. However, the *luxS* gene of *E. coli* DH5α was not affected by stationary-phase signals in this study (an average transcription level of *luxS* mRNA was detected for both the FCM and ACM samples, but *luxS* was not differentially expressed, since the expression ratios were about one in both the FCM and ACM microarray data sets); hence, the mode of AI-2 regulation appears to be degradation in the stationary phase. Interestingly, AI-1 of *Erwinia* and *Agrobacterium* sp., which are similar in structure to furanone, are subject to degradation by lactonase (AiiA) produced by *Bacillus cereus* (9).

Although indole has been reported to be a possible signal
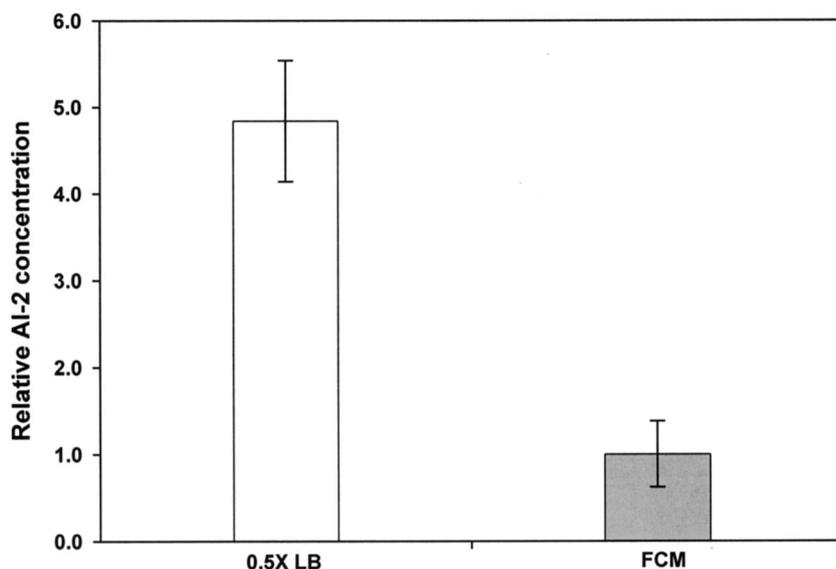
organic phase and water phase could be further purified with chromatography. Then each component may be tested for its effect on expression of the above genes.

REFERENCES

1. **Baca-DeLancey, R. R., M. M. T. South, X. Ding, and P. N. Rather.** 1999. *Escherichia coli* genes regulated by cell-to-cell signaling. Proc. Natl. Acad. Sci. USA **96:**4610–4614.
2. **Bassler, B. L.** 1999. How bacteria talk to each other: regulation of gene expression by quorum sensing. Curr. Opin. Microbiol. **2:**582–587.
3. **Bassler, B. L., M. Wright, and M. R. Silverman.** 1994. Multiple signaling systems controlling expression of luminescence in *Vibrio harveyi*: sequence and function of genes encoding a second sensory pathway. Mol. Microbiol. **13:**273–286.
4. **Beck von Bodman, S., D. R. Majerczak, and D. L. Coplin.** 1998. A negative regulator mediates quorum-sensing control of exopolysaccharide production in *Pantoea stewartii* subsp. *stewartii*. Proc. Natl. Acad. Sci. USA **95:**7687–7692.
5. **Cao, J.-G., and E. A. Meighen.** 1989. Purification and structural identification of an autoinducer for the luminescence system of *Vibrio harveyi*. J. Biol. Chem. **264:**21670–21676.
6. **Davies, D. G., M. R. Parsek, J. P. Pearson, B. H. Iglewski, J. W. Costerton, and E. P. Greenberg.** 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science **280:**295–298.
7. **DeLisa, M. P., J. J. Valdes, and W. E. Bentley.** 2001. Quorum signaling via AI-2 communicates the "Metabolic Burden" associated with heterologous protein production in *Escherichia coli*. Biotechnol. Bioeng. **75:**439–450.
8. **DeLisa, M. P., C.-F. Wu, L. Wang, J. J. Valdes, and W. E. Bentley.** 2001. DNA microarray-based identification of genes controlled by autoinducer 2-stimulated quorum sensing in *Escherichia coli*. J. Bacteriol. **183:**5239–5247.
9. **Dong, Y.-H., J.-L. Xu, X.-Z. Li, and L.-H. Zhang.** 2000. AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. Proc. Natl. Acad. Sci. USA **97:**3526–3531.
10. **Fawcett, P., P. Eichenberger, R. Losick, and P. Youngman.** 2000. The transcriptional profile of early to middle sporulation in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **97:**8063–8068.
11. **Greenberg, E. P., J. W. Hastings, and S. Ulitzur.** 1979. Induction of luciferase synthesis in *Beneckea harveyi* by other marine bacteria. Arch. Microbiol. **120:**87–91.
12. **Helmann, J. D., M. F. W. Wu, P. A. Kobel, F.-J. Gamo, M. Wilson, M. M. Morshedi, M. Navre, and C. Paddon.** 2001. Global transcriptional response of *Bacillus subtilis* to heat shock. J. Bacteriol. **183:**7318–7328.
13. **Hentzer, M., K. Riedel, T. B. Rasmussen, A. Heydorn, J. B. Andersen, M. R. Parsek, S. A. Rice, L. Eberl, S. Molin, N. Hoiby, S. Kjelleberg, and M. Givskov.** 2002. Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. Microbiology **148:**87–102.
14. **Jayaraman, A., A. K. Sun, and T. K. Wood.** 1998. Characterization of axenic *P. fragi* and *E. coli* biofilms for corrosion inhibition of SAE 1018 steel. J. Appl. Microbiol. **84:**485–492.
15. **Lithgow, J. K., V. E. Danino, J. Jones, and J. A. Downie.** 2001. Analysis of N-acyl homoserine-lactone quorum-sensing molecules made by different strains and biovars of *Rhizobium leguminosarum* containing different symbiotic plasmids. Plant Soil **232:**3–12.
16. **Miller, M. B., and B. L. Bassler.** 2001. Quorum sensing in bacteria. Annu. Rev. Microbiol. **55:**165–199.
17. **Park, S., P. M. Wolanin, E. A. Yuzbashyan, P. Silberzan, J. B. Stock, and R. H. Austin.** 2003. Motion to form a quorum. Science **301:**188.
18. **Pratt, L. A., W. Hsing, K. E. Gibson, and T. J. Silhavy.** 1996. From acids to *osmZ*: Multiple factors influence SYnthesis of the OmpF and OmpC Porins in *Escherichia coli*. Mol. Microbiol. **20:**911–917.
19. **Prigent-Combaret, C., O. Vidal, C. Dorel, and P. Lejeune.** 1999. Abiotic

20. surface sensing and biofilm-dependent regulation of gene expression in *Escherichia coli*. J. Bacteriol. **181:**5993–6002.
20. **Ren, D., L. A. Bedzyk, S. M. Thomas, R. W. Ye, and T. K. Wood.** Gene expression in *Escherichia coli* biofilms. Appl. Microbiol. Biotechnol., in press.
21. **Ren, D., J. J. Sims, and T. K. Wood.** 2001. Inhibition of biofilm formation and swarming of *Escherichia coli* by (5*Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(5*H*)-furanone. Environ. Microbiol. **3:**731–736.
22. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
23. **Schembri, M. A., K. Kjærgaard, and P. Klemm.** 2003. Global gene expression in *Escherichia coli* biofilms. Mol. Microbiol. **48:**253–267.
24. **Sperandio, V., C. C. Li, and J. B. Kaper.** 2002. Quorum-sensing *Escherichia coli* regulator A: a regulator of the LysR family involved in the regulation of the locus of enterocyte effacement pathogenicity island in enterohemorrhagic *E. coli*. Infect. Immun. **70:**3085–3093.
25. **Sperandio, V., J. L. Mellies, W. Nguyen, S. Shin, and J. B. Kaper.** 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.
26. **Sperandio, V., A. G. Torres, J. A. Giron, and J. B. Kaper.** 2001. Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. J. Bacteriol. **183:**5187–5197.
27. **Sperandio, V., A. G. Torres, B. Jarvis, J. P. Nataro, and J. B. Kaper.** 2003. Bacteria-host communication: the language of hormones. Proc. Natl. Acad. Sci. USA **100:**8951–8956.
28. **Stanley, N. R., R. A. Britton, A. D. Grossman, and B. A. Lazazzera.** 2003. Identification of catabolite repression as a physiological regulator of biofilm formation by *Bacillus subtilis* by use of DNA microarrays. J. Bacteriol. **185:**1951–1957.
29. **Stintzi, A., K. Evans, J.-M. Meyer, and K. Poole.** 1998. Quorum-sensing and siderophore biosynthesis in *Pseudomonas aeruginosa*: *lasR/lasI* mutants exhibit reduced pyoverdine biosynthesis. FEMS Microbiol. Lett. **166:**341–345.
30. **Surette, M. G., and B. L. Bassler.** 1998. Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA **95:**7046–7050.
31. **Surette, M. G., M. B. Miller, and B. L. Bassler.** 1999. Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. Proc. Natl. Acad. Sci. USA **96:**1639–1644.
32. **Wang, D., X. Ding, and P. N. Rather.** 2001. Indole can act as an extracellular signal in *Escherichia coli*. J. Bacteriol. **183:**4210–4216.
33. **Wei, Y., J.-M. Lee, C. Richmond, F. R. Blattner, J. A. Rafalski, and R. A. Larossa.** 2001. High-density microarray-mediated gene expression profiling of *Escherichia coli*. J. Bacteriol. **183:**545–556.
34. **Whiteley, M., M. G. Bangera, R. E. Bumgarner, M. R. Parsek, G. M. Teltzel, S. Lory, and E. P. Greenberg.** 2001. Gene expression in *Pseudomonas aeruginosa* biofilms. Nature **413:**860–864.
35. **Wilson, M., J. DeRisi, H.-H. Kristensen, S. Imboden, S. Rane, P. O. Brown, and G. K. Schoolnik.** 1999. Exploring drug-induced alterations in gene expression in *Mycobacterium tuberculosis* by microarray hybridization. Proc. Natl. Acad. Sci. USA **96:**12833–12838.
36. **Withers, H. L., and K. Nordström.** 1998. Quorum-sensing acts at initiation of chromosomal replication in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **95:**15694–15699.
37. **Xavier, K. B., and B. L. Bassler.** 2003. LuxS quorum sensing: more than just a numbers game. Curr. Opin. Microbiol. **6:**191–197.
38. **Ye, R. W., W. Tao, L. Bedzyk, T. Young, M. Chen, and L. Li.** 2000. Global gene expression profiles of *Bacillus subtilis* grown under anaerobic conditions. J. Bacteriol. **182:**4458–4465.
39. **Ye, R. W., T. Wang, L. Bedzyk, and K. M. Croker.** 2001. Application of DNA microarrays in microbial systems. J. Microbiol. Methods **47:**257–272.
40. **Zheng, M., X. Wang, L. J. Templeton, D. R. Smulski, R. A. LaRossa, and G. Storz.** 2001. DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. J. Bacteriol. **183:**4562–4570.