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Research Article

A Whole-Cell Biosensor for Point-of-Care Detection of Waterborne Bacterial Pathogens

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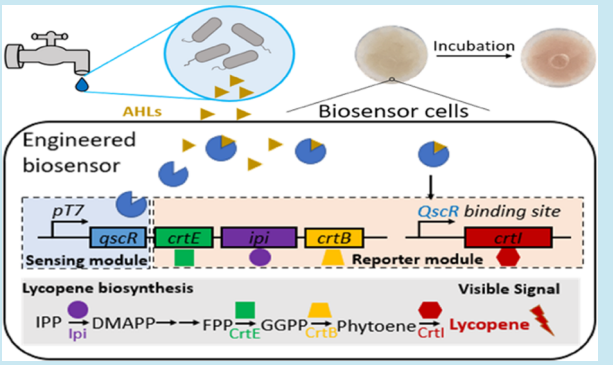
ABSTRACT: Water contamination by pathogenic bacteria is a major public health concern globally. Monitoring bacterial contamination in water is critically important to protect human health, but this remains a critical challenge. Engineered whole-cell biosensors created through synthetic biology hold great promise for rapid and cost-effective detection of waterborne pathogens. In this study, we created a novel whole-cell biosensor to detect water contamination by *Pseudomonas aeruginosa* and *Burkholderia pseudomallei*, which are two critical bacterial pathogens and are recognized as common causative agents for waterborne diseases. The biosensor detects the target bacterial pathogens by responding to the relevant quorum sensing signal molecules. Particularly, this study constructed and characterized the biosensor on the basis of the QscR quorum sensing signal system for the first time. We first designed and constructed a QscR on the basis of the sensing module in the *E. coli* host cell and integrated the QscR sensing module with a reporting module that expressed an enhanced green fluorescent protein (EGFP). The results demonstrated that the biosensor had high sensitivity in response to the quorum sensing signals of the target bacterial pathogens. We further engineered a biosensor that expressed a red pigment lycopene in the reporting module to produce a visible signal readout for the pathogen detection. Additionally, we investigated the feasibility of a paper-based assay by immobilizing the lycopene-based whole-cell biosensor on paper with the aim to build a prototype for developing portable detection devices. The biosensor would provide a simple and inexpensive alternative for timely and point-of-care detection of water contamination and protect human health.



KEYWORDS: *waterborne bacterial pathogens, whole-cell biosensor, quorum sensing, synthetic biology*

INTRODUCTION

Monitoring microbial contamination in water is becoming vitally important to inform consumers about water safety, identify source water problems, and facilitate decision-making to minimize human health risks. Around 10% of the world's total disease burden is attributable to unsafe potable water,[1] and global surveillance of waterborne pathogens that cause infectious diseases is of urgent need to protect human health. Early and point-of-care detection of bacterial pathogen contamination in water allows prompt disinfection, efficient control, and treatment of infection, but this remains a critical challenge.[2−4] Conventional detection methods based on culturing and staining are inherently time-consuming and labor intensive and do not provide timely results for monitoring contamination.[5] The advanced molecular approaches, such as nucleic acid-based and antibody-based assays, could achieve a relatively fast detection of target pathogens with high sensitivity. However, the molecular methods usually require sample preprocessing, specialized equipment, expensive reagents, and trained technical personnel, which prevents wide implementation of these alternatives especially in resource-limited settings.[6,7] Therefore, it is critically important to develop an affordable, easy-to-use, and rapid assay alternative for monitoring pathogen contamination in susceptible water systems.

With recent advances in synthetic biology, engineered whole-cell biosensors have emerged as a new platform for various environmental and biomedical applications, and they hold promise to provide novel diagnostic devices for easily deployed point-of-care testing.[8−10] Whole-cell biosensors use microbial cells as the receptor and transducer to respond to the presence of chemicals or physiological stresses and produce detectable output signals. The cells are typically genetically engineered and are composed of a sensing module and a reporting module, which allows for detection, recording, and quantification of the target analyte.[11,12] The main advantages that whole-cell

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microbial biosensors have over chemical or physical analytical techniques are low cost, portability, and environmental compatibility. Microorganisms can grow rapidly in relatively inexpensive media and produce necessary biological elements (*e.g.*, transcriptional factors for sensing modules, signal molecules for reporting modules), leading to low reagent costs. Moreover, unlike the conventional detection methods requiring large and complex analytical instruments (*e.g.*, chromatograph), whole-cell biosensors can detect a bioavailable substance directly from the environment or organisms, providing a point-of-care detection method. Direct detection of the target analytes without complex sample preparation is also an advantage of whole-cell biosensors compared to cell-free biosensors. Several cell-free biosensors have been recently developed with high specificity, flexibility, and short reaction time for bacteria detection. However, these systems often require sample preparation and expensive cell-free expression. For example, a cell-free biosensor for *P. aeruginosa* detection created by Wen *et al.* required bacteria cell extraction from infected respiratory samples and relied on an expensive *Escherichia coli* cell-free expression system from commercial vendors.[13] In another example, the cell-free sensor for analyzing gut microbiota and disease associated host biomarkers developed by Takahashi *et al.* required RNA extraction from samples and an isothermal RNA amplification step.[14] Recent research efforts have demonstrated the successful construction of whole-cell biosensors by using synthetic biology approaches for applications including environmental pollutant detection,[15−17] disease treatment,[18] and the production of biomedical commodities.[19] Various engineered whole-cell biosensing systems have been developed on the basis of diverse quorum sensing systems in nature, including the agr,[20] ahl,[21] cep,[22] cin,[23,24] cqs-lux,[25,26] cvi,[27] esa,[28] fsr,[29] las,[30] lux,[31] phz,[32] rhl,[33] rpa,[34] and tra quorum sensing systems.[35] For example, Holowko *et al.*[25] engineered an *E. coli* biosensor to detect and kill *Vibrio cholerae* based on a cholera autoinducer-1 (CAI-1) and the cqs-lux system, which could be potentially used for preventing and treating cholera disease.[26] Lubkowicz *et al.* created a biosensor to detect *Staphylococcus aureus* through engineering *Lactobacillus reuteri* with the agr system to sense autoinducer peptide-I, a quorum sensing molecule produced by *Staphylococcus sp.* during pathogenesis.[20] Noticeably, the previous studies have been primarily focused on biomedical applications while the potential of whole-cell biosensors for environmental applications, such as detecting waterborne pathogens, has been underexplored.

In this study, we created a novel whole-cell biosensor to detect water contamination by the pathogenic bacteria *Pseudomonas aeruginosa* and *Burkholderia pseudomallei*. *P. aeruginosa* and *B. pseudomallei* are two critical bacterial pathogens in contaminated water and have been recognized as common causative agents for waterborne diseases.[36−38] *P. aeruginosa* is an opportunistic human pathogen and has emerged as an important cause of various infectious diseases worldwide.[39] The pathogen caused an estimated 32,600 infections among hospitalized patients and 2,700 deaths in the United States in 2017.[39,40] *P. aeruginosa* is capable of colonizing a wide variety of aquatic environments including wet and moist niches in man-made water distribution systems. It is more likely to exist as a biofilm than in planktonic form in water systems, and its biofilms are commonly found in plumbing fixtures.[41,39] For example, *P. aeruginosa* was recovered by cultivation from drain biofilm swabs in 16 of 28 faucets (57%), while it was detected in the water phase in only 2 of 28 faucets (7%) during a post-outbreak investigation of *Pseudomonas aeruginosa* faucet contamination.[42] Microorganisms in biofilms are problematic as they are resistant to disinfection and environmental stress. Bacterial pathogens can be released from the biofilm as a result of physical disturbance or active detachment to bulk water, which poses a threat to human health.[43] Because of the dominant biofilm colonization, faucets, sinks, shower drains, drinking water fountains, and connection plumbing are recognized as the main reservoirs for *P. aeruginosa*, and the common route of infection is exposure to contaminated water including drinking, showering, and contact with objects rinsed with contaminated tap water.[44−47] For example, it has been found that up to 42% of *P. aeruginosa* that caused invasive infections in intensive care units (ICUs) originated from water,[48] and the bacteria were also the principal source of folliculitis and ear infections, for which over 80% of the cases were potable water derived.[49−51] As for *B. pseudomallei*, it is a highly pathogenic bacterium that causes an estimated 165,000 cases of melioidosis per year worldwide and is also classified as a biothreat agent.[52,53] Similar to *P. aeruginosa*, *B. pseudomallei* is often found in water systems, and a number of melioidosis cases were linked to a contaminated water supply.[54] However, it is currently difficult to detect *P. aeruginosa* and *B. pseudomallei* in water samples in a timely and routine manner, as conventional laboratory methods are time-consuming and expensive. Therefore, the whole-cell biosensor developed in this study aimed to address the above-mentioned challenges by providing a new approach for timely and point-of-care detection of the bacterial pathogen contamination in water.

The biosensor in this study detects the target bacterial pathogens by responding to the relevant quorum-sensing molecules. Particularly, this study demonstrated for the first time the construction of a whole-cell biosensor based on the QscR quorum sensing system originally found in *P. aeruginosa*. Quorum sensing is a process of cell−cell communication that allows bacteria to detect and respond to cell population density by gene regulation.[55] *P. aeruginosa* employs *N*-acylhomoserine lactone (AHL) as their command language to coordinate population behavior during invasion and colonization.[56] There is a complete quorum sensing regulatory circuit LasI−LasR in *P. aeruginosa*. The LasI synthase constitutively produces the signal molecule *N*-3-oxododecanoyl-homoserine lactone ($3OC_{12}$-HSL), which binds the transcriptional regulator LasR and the resultant LasR−$3OC_{12}$-HSL complex activates gene transcription.[57] Several biosensors based on the LasI−LasR system have been developed for specific detection of *N*-dodecanoyl-homoserine lactone (C_{12}-HSL), *N*-tetradecanoyl-homoserine lactone (C_{14}-HSL), and their 3-oxo derivatives.[13,30,33,58,59] Struss *et al.* created a paper strip biosensor based on the LasR system to detect C_{12}-HSL at a concentration down to 1.0×10^{-8} M,[30] and Kumari *et al.* used a whole-cell LasR biosensing system to detect C_{12}-HSL and C_{14}-HSL in saliva and stool samples with a detection limit of 1.0×10^{-9} M.[33] However, the LasR biosensors do not detect any 3-hydroxy AHLs.[58,59] Besides the LasI−LasR system, QscR is a LasR homologue for which there is no cognate AHL synthase. As a transcriptional activator, QscR regulates the expression of target genes when it binds to the LasI−LasR cognate signal molecule $3OC_{12}$-HSL. QscR responds to $3OC_{12}$-HSL in almost exactly the same way as LasR, but QscR can also respond to 3-hydroxy AHL.[60,61] The ability of QscR to respond to 3-hydroxy AHL is advantageous for creating biosensors for water contamination monitoring, because some waterborne bacterial pathogens produce 3-hydroxy AHLs as the main quorum sensing signals.

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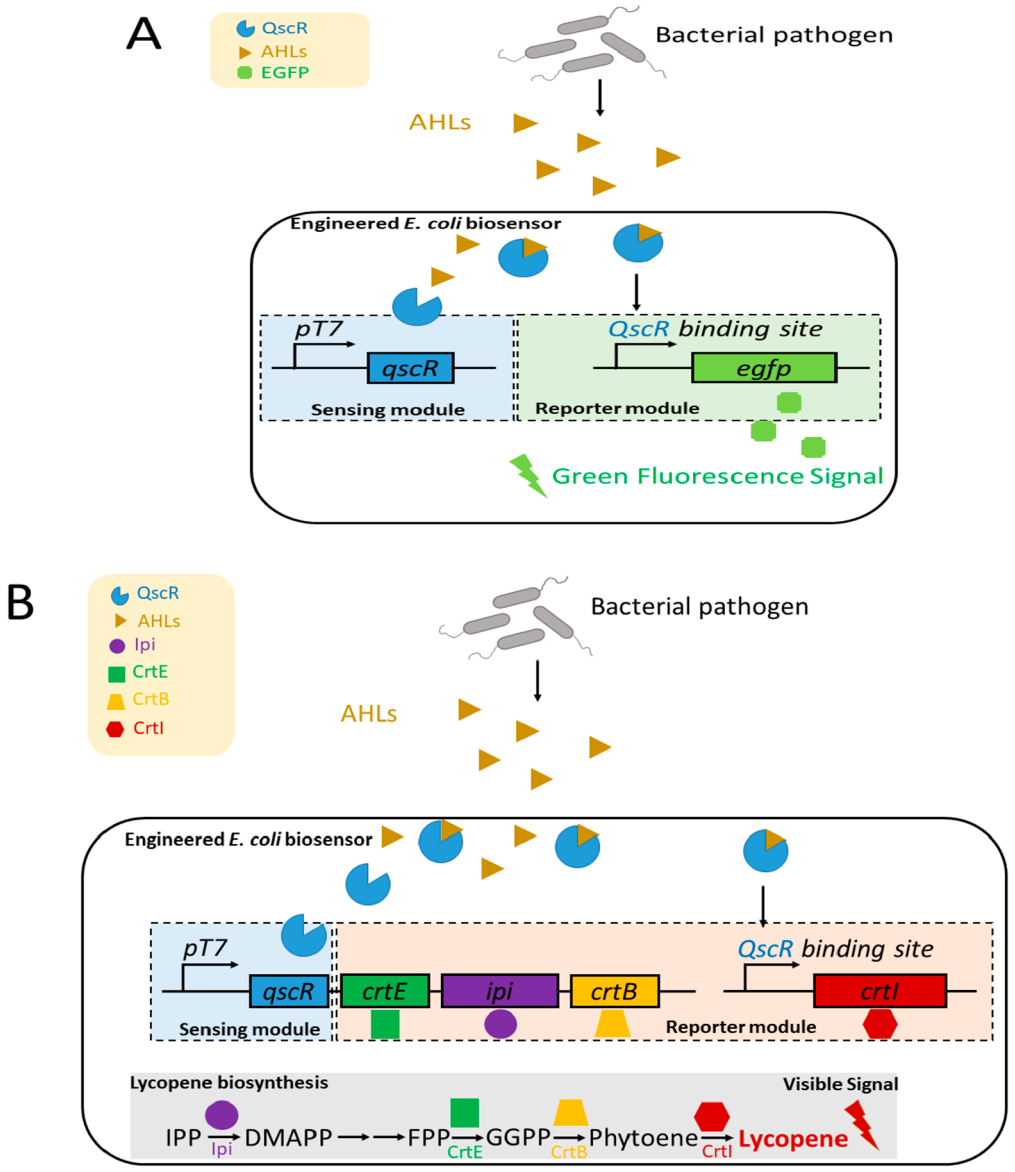

Figure 1. Schematic of engineered whole-cell biosensor containing the QscR sensing module coupled with enhanced green fluorescence protein (EGFP) reporting module (A) or lycopene reporting module (B). Abbreviations used are as follows: QscR, a transcriptional activator protein from *P. aeruginosa*; AHLs, *N*-acylhomoserine lactone; CrtE, geranylgeranyl pyrophosphate synthase; CrtB, phytoene synthase; CrtI, lycopene synthase; Ipi, isopentenyl pyrophosphate isomerase; IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate, FPP, farnesyl diphosphate; GGPP, geranylgeranyl pyrophosphate.

For example, the bacterial pathogen *B. pseudomallei* has been found to produce *N*-3-hydroxydecanoyl HSL ($3OHC_{10}$-HSL) as one of the main signal molecules.[62] Therefore, by exploring the function of the QscR quorum-sensing system, we designed and engineered a whole-cell biosensor to detect the bacterial pathogens.

Specifically, this paper discusses the results of the construction and characterization of a QscR-based sensing module in an *E. coli* host cell coupled to two types of readouts, green fluorescence signal and red color visible signal, respectively (Figure 1). We first integrated the QscR sensing module and the reporting module that expressed an enhanced green fluorescent protein (EGFP). The results demonstrated that the biosensor had high sensitivity in response to the quorum-sensing molecules of the target bacterial pathogens. We further engineered a biosensor that expressed a red pigment lycopene in the reporting module to produce a visible signal readout for the pathogen detection. Additionally, we investigated the feasibility of a paper-based assay by immobilizing the whole-cell biosensor on paper with the aim to build a prototype of portable detection devices. We envision that the biosensor will provide a simple and inexpensive alternative for timely detection of the bacterial pathogens in water systems, enabling point-of-care monitoring of water contamination to protect human health.

RESULTS AND DISCUSSION

Construction of a Functional Biosensor with Green Fluorescence Readout. As shown in Figure 1, the engineered biosensor is composed of two modules: the sensing module and reporting module. The sensing module expresses the transcription factor QscR, which can bind to the AHL molecules that

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are secreted from *P. aeruginosa* and *B. pseudomallei*. Then, the formation of QscR−AHL complex can bind to an inducible promoter, which leads to the activation of the reporting module. As for the reporting module, we first designed a green fluorescent protein-based system, in which the expression of EGFP would be induced by the QscR−AHL complex. The highly efficient expression and easy detection of the green fluorescence signal could facilitate the characterization and optimization of the biosensor system. The key transcription factor QscR was expressed under the control of the constitutive T7 promoter in the plasmid pET23b(+) (Figure S1). A PA1897 promoter was used as the inducible promoter to control the expression of EGFP. The PA1897 is adjacent to *qscR* on the *P. aeruginosa* genome and was reported to be a promoter region activated by the QscR−AHL complex.[60,63] The successful expression of QscR with the His-tag was confirmed by Western blotting, which showed a band at ∼25 kDa, corresponding to the reported molecular weight of the QscR monomer (Figure S2).[64]

Three synthetic AHLs (*i.e.*, $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL) were used to determine the functionality of the engineered biosensor by measuring the green fluorescence signal in response to the presence of these AHLs, respectively. $3OC_{12}$-HSL is the cognate AHL produced by *P. aeruginosa* while C_{10}-HSL and $3OHC_{10}$-HSL are the AHLs produced by *B. pseudomallei*. As reported in previous studies, $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL are the AHLs that interact with QscR and activate the response of the target genes.[61,65] In the presence of the synthetic AHL molecules, the engineered biosensor cells produced strong green fluorescence signals, while in the control condition without the AHL molecules, there was no green fluorescence signal observed (Figure 2). The results demonstrated that the engineered QscR sensing module was functional when coupled with the EGFP reporting module.

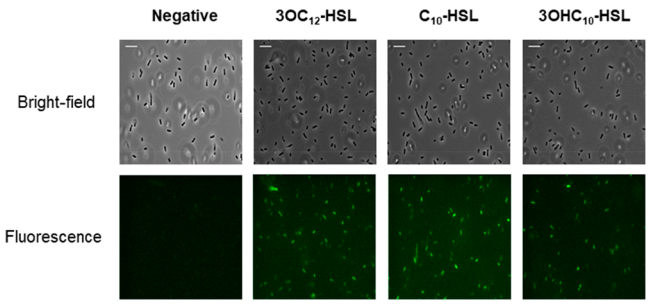

Figure 2. Fluorescence microscope photographs of the biosensor cells incubated with the synthetic AHL molecules $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL, respectively. Incubation without the addition of the AHLs was used as the negative control. The bright-field images showed the cell morphology of the biosensor cells, and the fluorescence images showed green fluorescence signals emitted from the cells. Scale bar: 5 μm.

Specificity and Sensitivity of the Engineered Biosensor. For on-site pathogen surveillance in water systems, it is important that the biosensor can detect the target AHL molecules at environmentally relevant concentrations with minimal cross-activation by AHLs from different bacterial species. To characterize the specificity of the engineered biosensor toward the target AHL molecules produced from *P. aeruginosa* and *B. pseudomallei*, we quantified the generation of EGFP fluorescence signal in the presence of different AHL molecules. Besides the AHLs ($3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL) produced from *P. aeruginosa* and *B. pseudomallei*, three additional AHL molecules, *N*-3-hydroxy-octanoyl homoserine lactone ($3OHC_8$-HSL), *N*-octanoyl homoserine lactone (C_8-HSL), and *N*-octanoyl homoserine lactone (C_6-HSL), were included in the batch experiments. The AHL molecule $3OHC_8$-HSL was chosen because it is the most common quorum-sensing molecule for regulating the biofilm formation of *Pseudomonas spp*. except *P. aeruginosa*, which does not produce hydroxyl-HSL such as $3OHC_8$-HSL.[66−68] C_8-HSL and C_6-HSL are the AHLs produced by *Burkholderia cenocepacia*, which was found to not only inhabit the same environmental niches as *P. aeruginosa* but also form mixed biofilms with *P. aeruginosa* in the lungs of cystic fibrosis patients.[22,69] As shown in Figure 3, the

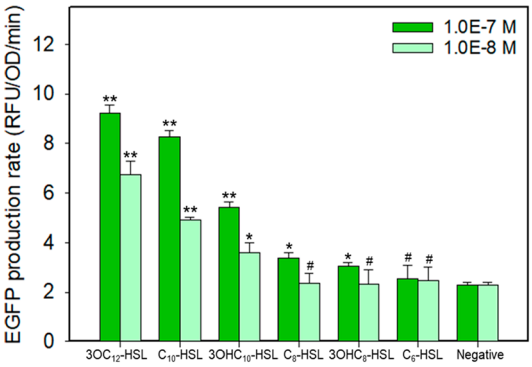

Figure 3. Response of the biosensor toward six synthetic AHLs. The biosensor with green fluorescence readout was induced in the presence of each AHL at 1.0×10^{-7} and 1.0×10^{-8} M for 3 h, and the EGFP production rates were quantified. The biosensor without synthetic AHL molecules was used as the negative control. Student's *t* tests between each AHL and the negative control were performed, and statistically significant differences are indicated as follows: $^{**}P \leq 0.0001$, $^{*}P \leq 0.005$, and $\#P > 0.1$. The results are the means of triplicate experiments; error bars indicate standard deviations.

biosensor had the highest EGFP production rate in the presence of $3OC_{12}$-HSL at the concentrations of 1.0×10^{-8} and 1.0×10^{-7} M, suggesting that the biosensor had a strong response to the cognate AHL from *P. aeruginosa*. The EGFP production rates induced by C_{10}-HSL and $3OHC_{10}$-HSL were also substantial at both concentrations and were significantly higher than that induced by C_8-HSL, $3OHC_8$-HSL, C_6-HSL, or the negative control ($P \leq 0.05$, Table S1). In addition, EGFP production (RFU/OD) of the biosensors induced by $3OC_{12}$-HSL, C_{10}-HSL, or $3OHC_{10}$-HSL increased quickly after the addition of AHLs, which was 2−3-fold higher than that of negative control within 3 h (Figures S3 and S4). The results showed that the engineered QscR sensing module had a preferential interaction with $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL over the other AHLs, suggesting that the biosensor could target *P. aeruginosa* and *B. pseudomallei* specifically.

We then characterized the sensitivity of the engineered biosensor by quantifying the EGFP production rates in response to a range of concentrations of $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL, respectively. As shown in Figure 4, the EGFP production rates started to increase when the concentration of $3OC_{12}$-HSL, C_{10}-HSL, or $3OHC_{10}$-HSL increased beyond 1.0×10^{-9} M, and the EGFP production peaked at a steady rate when $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL reached 1.0×10^{-7}, 5.0×10^{-7}, and 1.0×10^{-7} M, respectively. An empirical

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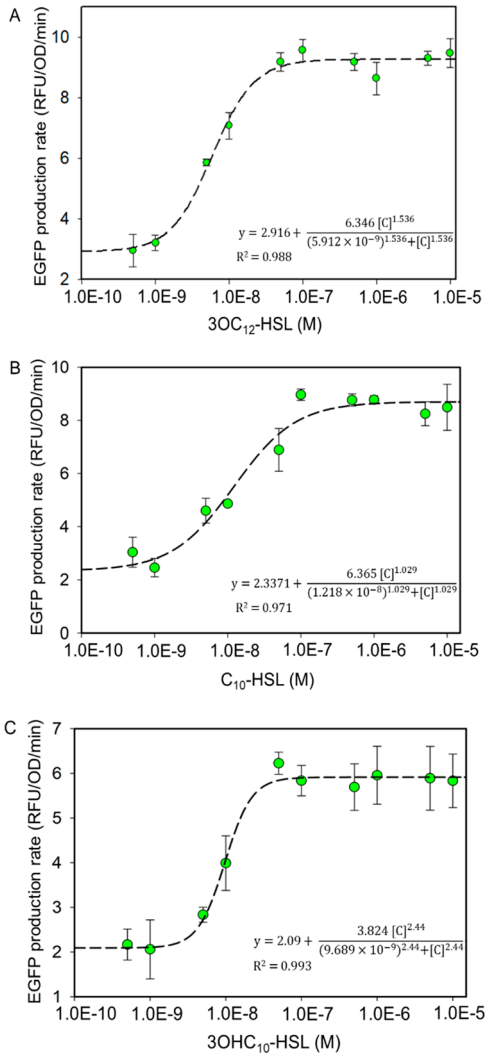

Figure 4. EGFP production rate at different concentrations of synthetic molecules $3OC_{12}$-HSL (A), C_{10}-HSL (B), or $3OHC_{10}$-HSL (C). The results are the means of triplicate experiments; error bars indicating standard deviations are not visible when smaller than the symbol size. The relationship between the EGFP production rate and the concentration of the synthetic AHL fits the Hill equation with an *R*-squared value above 0.9, as shown in the embedded equations.

mathematical model, Hill equation, was used to provide a quantitative description of the relationship between the EGFP production rate and AHL molecule concentration. The resulting best fit models provided EC_{50} values, which represent the concentration of AHL compounds required for half-maximal activation of the biosensor. The Hill equation showed that the biosensor was highly sensitive to the AHLs with EC_{50} values as low as 5.91×10^{-9} M for $3OC_{12}$-HSL, 1.22×10^{-8} M for C_{10}-HSL, and 9.69×10^{-9} M for $3OHC_{10}$-HSL. As reported in the literature, the concentration of $3OC_{12}$-HSL detected in the effluent from the *P. aeruginosa* biofilms was 1.4×10^{-8} M, and its concentration within the biofilm was over 600 μM.[70] The concentrations of $3OC_{12}$-HSL detected in the supernatant of planktonic cultures are often estimated to be between 1.0×10^{-8} and 5.0×10^{-6} M.[57,71] The biosensor had an EC_{50} value of 5.91×10^{-9} M for $3OC_{12}$-HSL, indicating the feasibility of the biosensor in detecting the target AHL molecule. It was also noted that the maximum EGFP production rates of the biosensor for $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL were 9.26, 8.70, and 5.91 RFU/OD/min, respectively. These results suggested that the engineered biosensor was most responsive to the cognate $3OC_{12}$-HSL with the lowest EC_{50} value and the highest EGFP production rate while it showed a bit lower sensitivity toward C_{10}-HSL and $3OHC_{10}$-HSL.

To evaluate the potential matrix effect of tap water on the performance of the engineered biosensor, we further characterized the dose−response curves of the biosensor in tap water samples spiked with $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL, respectively. The biosensor maintained EC_{50} values of 7.634×10^{-9}, 1.469×10^{-8}, and 9.927×10^{-9} M for $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL, respectively, in the tap water matrix (Figure S5), which were comparable to the EC_{50} values obtained in LB medium. The results suggested that the biosensor was robust in the real tap water environment.

Detection of the Native AHLs Produced by Bacterial Pathogens. To determine the ability of the whole-cell biosensor to detect native AHL molecules produced from *P. aeruginosa* and *B. pseudomallei* in solution, the biosensor cells were incubated in the filtered culture solution of the bacterial pathogens. The organism *B. pseudomallei* is considered a Tier 1 Select agent according to the Centers for Disease Control and Prevention due to its severe threat to public health and safety.[72] *Burkholderia thailandensis*, a nonpathogenic bacterium closely related to *B. pseudomallei* with a homologous quorum sensing system, is a commonly used surrogate model organism to study quorum sensing of *B. pseudomallei*.[62,73,74] Therefore, our study used *P. aeruginosa* strain PAO1 and *B. thailandensis* strain AU13555 to determine the response of the biosensor toward native AHLs. As observed in the microscope images (Figure 5A), the biosensor cells showed a clear green fluorescence signal after being incubated in the filtered culture solutions of *P. aeruginosa* PAO1 and *B. thailandensis* AU13555, respectively. The EGFP production increased drastically upon induction by the two bacterial culture solutions and reached the plateau within 5 h, while the negative control did not show significant EGFP production in 24 h (Figure 5B). The EGFP production rates for *P. aeruginosa* and *B. thailandensis* culture solutions were 6.63 and 4.63 RFU/OD/min, respectively. For the *P. aeruginosa* culture solution, the biosensor EGFP production rate was higher than the half-maximal rate induced by synthetic $3OC_{12}$-HSL (*i.e.*, 4.63 RFU/OD/min), indicating that *P. aeruginosa* secreted a substantial amount of $3OC_{12}$-HSL in the culture. As for the *B. thailandensis* culture solution, the EGFP production rate could be attributed to the concentration levels of both C_{10}-HSL and $3OHC_{10}$-HSL. In all, the results provided solid evidence that the whole-cell biosensor was able to detect native AHLs produced from *P. aeruginosa* and *B. thailandensis*.

Construction and Characterization of the Lycopene-Based Biosensor for a Visible Readout. A visible readout signal would be advantageous and desirable for efficient on-site pathogen detection. To this end, we engineered a reporting module by introducing a lycopene synthesis pathway to replace the EGFP expression cassette. Lycopene is a naturally occurring brilliant red pigment synthesized by certain plants and microorganisms. Using lycopene as a visible readout does not need any reagent and advanced equipment, which is useful especially in resource-limited settings. Microbial contamination in water is a major public health problem in the underdeveloped areas, but there is a lack of capability to detect water contamination due to the high cost and technical challenges of the current detection methods.[1,75] A biosensor with visible readout could provide an affordable, easy-to-use diagnostic tool

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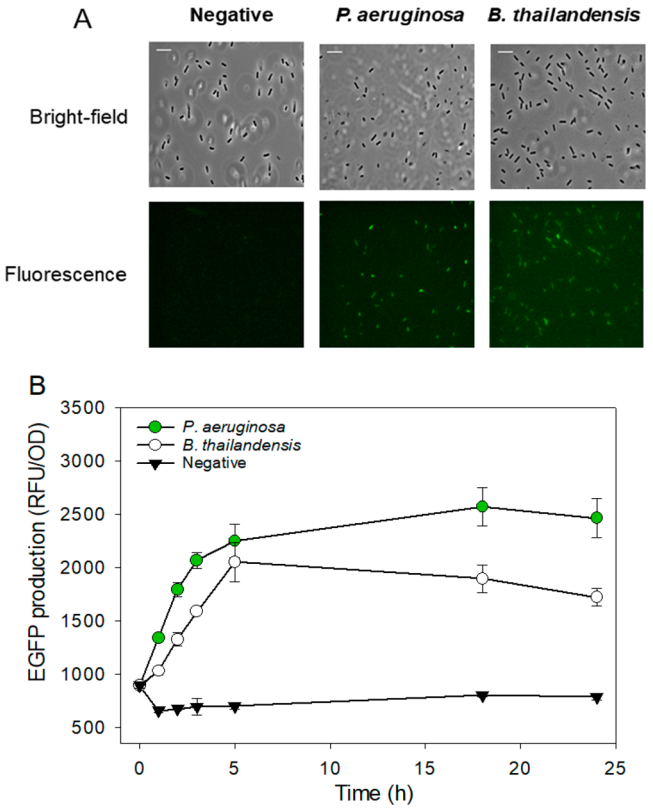

Figure 5. (A) Fluorescence microscope images of the biosensors incubated with a filtered culture solution of *P. aeruginosa* PAO1 or *B. thailandensis* AU13555 at a time point of 5 h. The incubation without a filtered culture solution was used as the negative control. The bright-field images showed the cell morphology of the biosensor cells, and the fluorescence images showed the green fluorescence signals emitted from the cells. Scale bar: 5 μm. (B) EGFP production rate of the biosensor in response to the filtered culture solutions. The results are the means of triplicate experiments; error bars indicating standard deviations are not visible when smaller than the symbol size.

for on-site monitoring and surveillance of waterborne pathogens. Regarding lycopene production, extensive efforts have been made in metabolic engineering to optimize lycopene biosynthesis.[76,77] The initial step of lycopene production, which involves the formation of farnesyl pyrophosphate (FPP) from the precursor dimethylallyl diphosphate (DMAPP), can occur through existing pathways in *E. coli*. Then, the expression of three exogenous biosynthetic genes (*crtE* (geranylgeranyl pyrophosphate synthase), *crtB* (phytoene synthase), and *crtI* (lycopene synthase)) would be sufficient for converting FPP to lycopene (Figure 1B).[77,78] In the present study, we introduced the lycopene production pathway into the biosensor host strain by expressing *crtE* and *crtB* under the T7 constitutive promoter in the plasmid pET23b-qscR-EIB (Figure S6) and expressing the gene *crtI* under the control of the inducible promoter PA1897 (which is activated by the QscR−AHL complex). An additional gene *ipi* encoding the *Erwinia herbicola* isopentenyl pyrophosphate isomerase was introduced to convert available isopentenyl pyrophosphate (IPP) to DMAPP precursor (Figure 1B) in order to increase the supply of DMAPP for the enhanced production of lycopene in *E. coli*.[79]

The engineered biosensor cells showed a red color upon induction by the synthetic AHL compounds $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL, respectively (Figure 6A), suggesting the establishment of a functional lycopene synthesis pathway in the reporting module. We then quantified the production of the lycopene signal induced by the synthetic AHLs. A quantitative absorbance-based method was used to measure lycopene production per cell (LPC), as detailed in the Supporting Information. We measured lycopene production induced by 2.0×10^{-9}, 5.0×10^{-8}, and 1.0×10^{-7} M $3OC_{12}$-HSL, C_{10}-HSL, or $3OHC_{10}$-HSL, respectively (Figure 6B). The lycopene production in the whole-cell biosensor reached around 0.3 LPC units in 8 h upon induction by $3OC_{12}$-HSL, C_{10}-HSL, or $3OHC_{10}$-HSL at 5.0×10^{-8} and 1.0×10^{-7} M, and the lycopene production showed no obvious difference between the AHL concentrations of 5.0×10^{-8} and 1.0×10^{-7} M during the incubation time frame. When induced by 2.0×10^{-9} M of each synthetic AHL molecule, the biosensor still exhibited a significant level of lycopene production though the yield was lower than that induced by higher concentrations of AHLs. These results demonstrated that the lycopene-based biosensor could provide a sensitive detection of all three AHLs ($3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL) at concentrations as low as 2.0×10^{-9} M, and the lycopene production could be visible in 8 h of incubation.

We next evaluated the ability of the lycopene-based biosensor to detect native AHL molecules produced from the two bacterial pathogens. The biosensor cells were incubated in a filtered culture solution from *P. aeruginosa* and *B. thailandensis*, respectively. The lycopene production level increased upon induction, demonstrating the ability of the biosensor to detect native AHL molecules secreted from the bacterial pathogens. Visible red colors in both incubations were observed after 8 h (Figure 7). It was noted that the lycopene production induced by the filtered culture solution was relatively low, which was unexpected on the basis of the performance of the biosensor with the synthetic AHL in the same time frame. One possible reason could be that the bacterial culture solutions contained some compounds that could potentially inhibit lycopene biosynthesis. Since lycopene biosynthesis required the production of three synthesis proteins (*i.e.*, CrtE, CrtB, and CrtI) and precursors in the *E. coli* host cell, the inhibitory effect from the potentially toxic compounds on lycopene production would be larger than that on the production of the fluorescence signal in the EGFP-based biosensor. Further study is needed to determine the effects of the bacterial culture solution on lycopene synthesis and improve the lycopene production in the biosensor.

Design of a Point-of-Care Paper-Based Assay. We attempted to design a simple paper-based assay with the whole-cell biosensor immobilized in the alginate hydrogel and spotted on paper. The biosensor cells were cultured in LB medium to an OD_{600} of 2.0, and an appropriate amount of cell pellets was mixed with alginate to generate a 10^9 cells per hydrogel spot on a filter paper with a diameter of 5 mm. One paper-based assay contained a biosensor spot and a negative control spot with *E. coli* cells containing the lycopene synthesis pathway as the reporting module but no QscR sensing module. The detection was performed by immerging the paper in a 5 mL solution containing synthetic $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL, respectively. A visible coral color was developed in the biosensor spot after 16 h of incubation with 5.0×10^{-8} M $3OC_{12}$-HSL, 1.0×10^{-7} M C_{10}-HSL, and 1.0×10^{-6} M $3OHC_{10}$-HSL, respectively, while the negative control spot showed a yellow color representing the grown *E. coli* control cells (Figure S7). It was noted that the immobilized biosensor did not show a clear red color when induced with a lower concentration (2.0×10^{-9}

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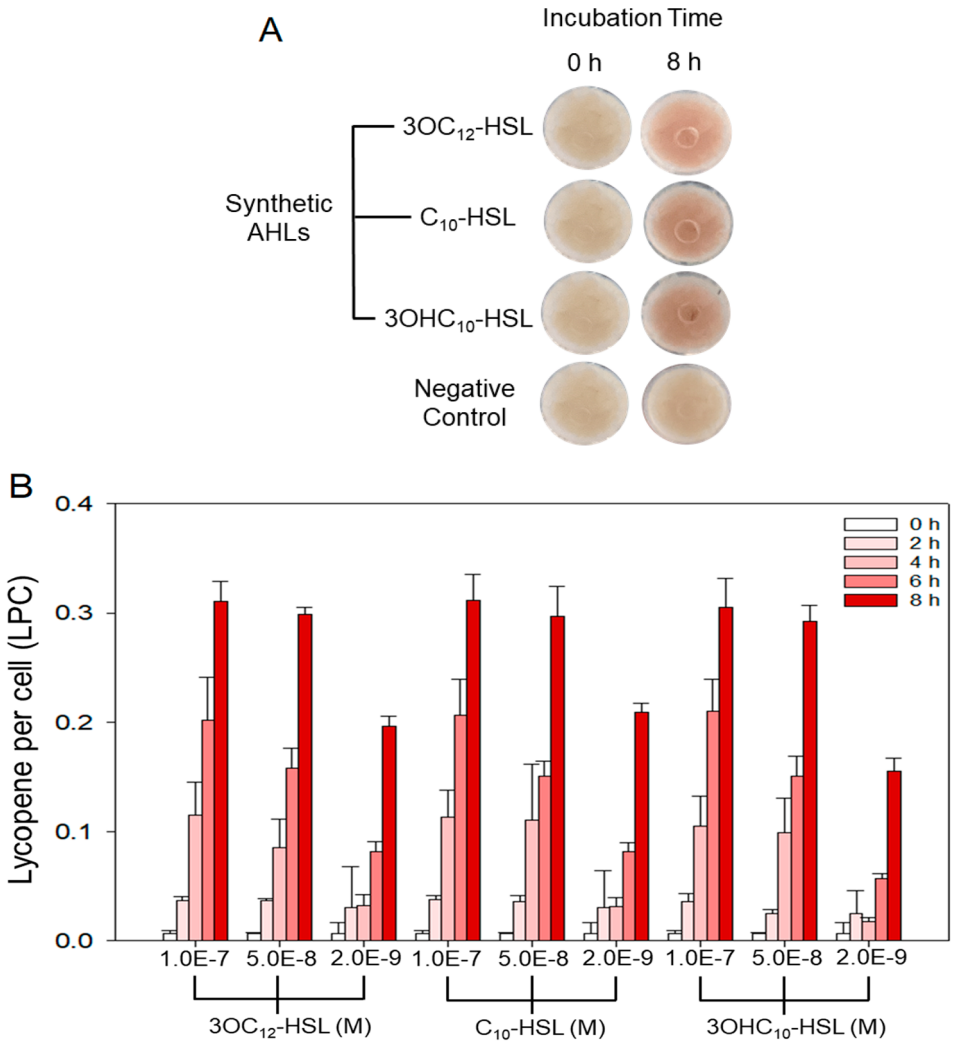

Figure 6. (A) Photographs of the lycopene-producing biosensors incubated with 1.0×10^{-7} M $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL, respectively, for 8 h. The incubation without the addition of synthetic AHL molecules was used as the negative control. (B) Lycopene production in the biosensor in response to synthetic AHL molecules $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL, respectively, at three different concentrations (1.0×10^{-7}, 5.0×10^{-8}, and 2.0×10^{-9} M). The results are the means of triplicate experiments; error bars indicated standard deviations.

M) for all three synthetic AHLs. There might be two possible reasons: (i) the hydrogel immobilization could have an influence on the mass transfer of the AHL molecules to the biosensor cells, leading to a lower yield of lycopene production compared to that from the suspended biosensor when treated with the same concentration of AHLs; (ii) the alginate hydrogel itself had a white color, which might dilute the red color of the lycopene. The results indicated the feasibility of the paper-based assay, and future work will focus on improving lycopene yield and optimizing the immobilization strategy to develop a more sensitive paper-based assay method.

MATERIALS AND METHODS

Strains, Medium, and Chemicals. *E. coli* strain BL21 (F− *ompT gal dcm lon* $hsdS_B(r_B^-m_B^-)$ λ(DE3 [*lacI lacUV5-T7p07 ind1 sam7 nin5*]) $[malB^+]_{K\text{-}12}(\lambda^S)$) was obtained from New England Biolabs (NEB) (Beverly, MA) and was used in this study as the host organism for the whole-cell biosensor designed in this study. *E. coli* TOP10 (Invitrogen) was used for gene cloning and manipulation. The *E. coli* cells were regularly grown in Luria−Bertani (LB) broth (tryptone 10 g/L, yeast extract 5 g/L, and sodium chloride 10 g/L purchased as premixed media from BD) at 37 °C, and 100 μg/mL ampicillin and 50 μg/mL kanamycin were added to the medium when required. Restriction enzymes, ligase, and molecular reagents for polymerase chain reaction (PCR) were obtained from NEB. All other general chemicals and medium components were supplied by Fisher Scientific (Pittsburgh, PA).

Homoserine Lactone Stock Solutions and Pathogenic Bacterial Strains. To investigate the response of the whole-cell biosensor toward the AHL molecules excreted from bacterial pathogens, both synthetic homoserine lactone signal compounds and opportunistic bacterial pathogens were used in this study. The synthetic homoserine lactones $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL (Cayman Chemical, MI) were dissolved in dimethyl sulfoxide (DMSO) to make a stock solution of 10 mM. Pathogenic bacterial strains *Pseudomonas aeruginosa* PAO1 and *Burkholderia thailandensis* AU13555 were used in this study. The culture supernatant was harvested by centrifugation when the cells were grown from OD_{600} 1 to OD_{600} 3 in LB broth at 30 °C and 250 rpm. The supernatant was further sterilized through filtration with a filter membrane (0.22 μm).

Constructions of Expression Plasmids. Information on plasmids in this study is summarized in Table S2. To construct

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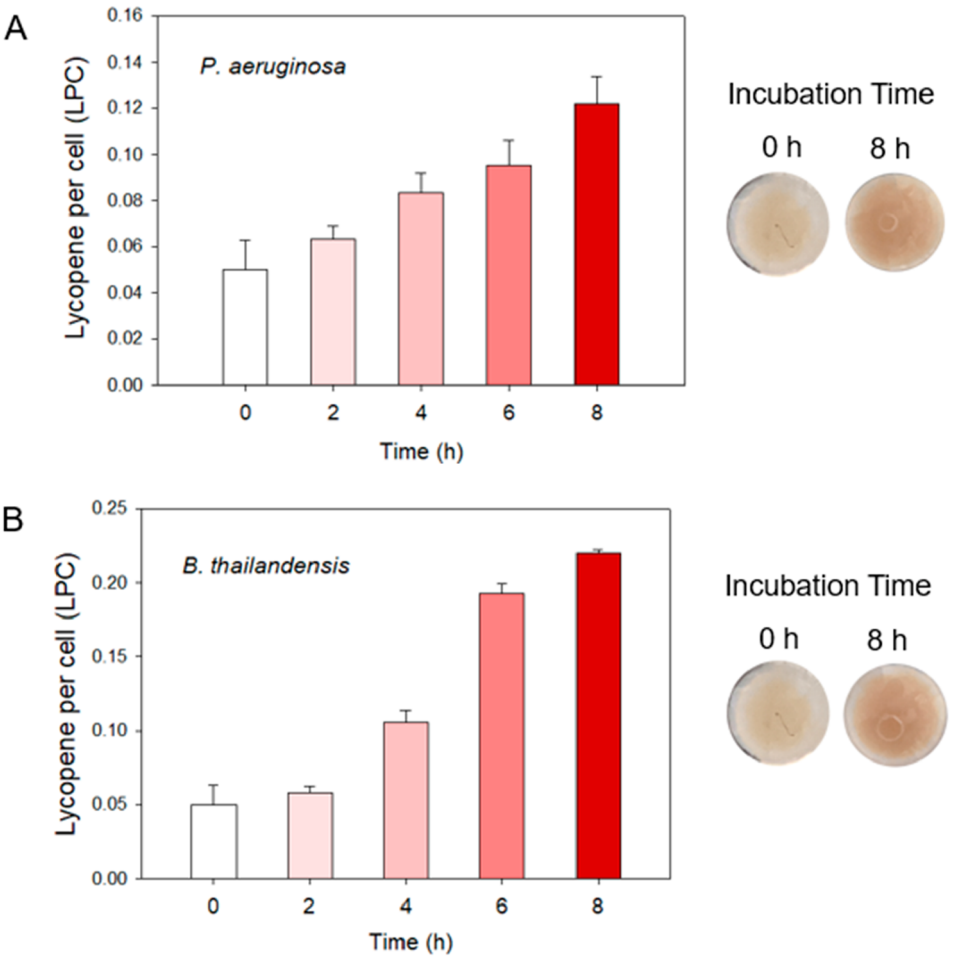

Figure 7. Lycopene production in the biosensors induced by the filtered culture solutions of *P. aeruginosa* (A) and *B. thailandensis* (B), respectively, for 8 h. The results are the means of triplicate experiments; error bars indicating standard deviations are not visible when smaller than the symbol size.

the QscR expression plasmid, the *qscR* open reading frame (ORF) was amplified from the *P. aeruginosa* PAO1 genome and cloned into pET23b(+) under a T7 constitutive promoter with a His_6-tag at the C terminus. The resulting plasmid was named pET23b-qscR. For the biosensor with the fluorescence signal in the reporting module, the EGFP sequence amplified from pEGFP-C1 and the QscR−AHL complex binding site and the PA1897 promoter were cloned into the pUC57kan plasmid, yielding the plasmid pUC57-egfp. The plasmids pET23b-qscR and pUC57-egfp were transformed into the *E. coli* BL21 strain yielding the whole-cell biosensor with green fluorescence signal. To construct the whole-cell biosensor with a lycopene biosynthesis pathway, *crtE* (geranylgeranyl pyrophosphate synthase), *ipi* (isopentenyl pyrophosphate isomerase), and *crtB* (phytoene synthase) amplified from pAC-LYC were subcloned into pET23b-qscR, yielding the plasmid pET23b-qscR-EIB.[80] To couple lycopene production to the activation of the QscR receptor, *crtI* (lycopene synthase) amplified from pAC-LYC and the promoter PA1897 were cloned into pUC57kan, yielding the plasmid pUC57-crtI. The plasmids pET23b-qscR-EIB and pUC57-crtI were transformed into the *E. coli* BL21 strain forming a biosensor with the red pigment lycopene as the reporting signal. The *lac* operon in pUC57kan was removed to prevent leaky expression. All the primers used in this study were purchased from Integrated DNA Technologies (Coralville, IA) and are listed in Table S2.

Western Blot Analysis. Proteins extracted from 1×10^9 cells were resolved by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the gel was transferred to a polyvinylidene difluoride membrane. The membrane was blocked in 5% nonfat milk at room temperature for 1 h, followed by incubation with anti-His (GenScript, 6G2A9, 1:2000) or anti-GAPDH (Abcam, GA1R, 1:5000) primary antibody at room temperature for 2 h. The membrane was washed and probed by antimouse HRP (Abcam, ab6728, 1:10 000) secondary antibody. Signals were developed using Pierce ECL Western Blotting Substrate (Thermo Scientific) and detected by a C400 Bioanalytical Imager (Azure Biosystems).

Characterization of the Biosensor with the EGFP Reporting Module. Single colonies of the biosensor with the EGFP reporting module were inoculated into 5 mL of LB broth with 100 μg/mL ampicillin and 50 μg/mL kanamycin and incubated overnight in a shaking incubator at 37 °C and 250 rpm. The culture was diluted to an OD_{600} of ∼0.002 and incubated further to an OD_{600} of 0.3 under the same condition. Cell culture was then transferred into a test tube in triplicate aliquots for induction with six synthetic AHLs ($3OC_{12}$-HSL, C_{10}-HSL, $3OHC_{10}$-HSL, C_8-HSL, $3OHC_8$-HSL, and C_6-HSL, respectively) at two concentrations (1.0×10^{-8} and 1.0×10^{-7} M) for a specificity test. The two concentrations of AHLs were chosen on the basis of (i) the average range of AHL concentrations in water environments as reported in the

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literature (1.0×10^{-8} to 5.0×10^{-6} M for $3OC_{12}$-HSL[57,70,71]) and (ii) the results from the dose−response curves for $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL, where the EGFP production rates all stabilized at the concentration level above 1.0×10^{-7} M. Therefore, we chose 1.0×10^{-8} M as the low concentration and 1.0×10^{-7} M as the high concentration in the specificity test. Student's t tests were performed to determine the significant differences in EGFP production rates of the biosensors induced by each AHL and the negative control. To examine the sensitivity of the biosensor, the cell culture was induced by $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL, respectively, at varied concentrations (5.0×10^{-10}, 1.0×10^{-9}, 5.0×10^{-9}, 1.0×10^{-8}, 5.0×10^{-8}, 1.0×10^{-7}, 5.0×10^{-7}, 1.0×10^{-6}, 5.0×10^{-6}, and 1.0×10^{-5} M). All the tubes were incubated at 37 °C and 250 rpm, and the aliquots of the cultures (300 μL) were withdrawn at intervals of 30 min for a total experimental time of 3 h. To analyze the matrix effect, 0.3 mL of actual tap water samples spiked with $3OC_{12}$-HSL, C_{10}-HSL, or $3OHC_{10}$-HSL was added in triplicate to culture tubes containing 2.7 mL of cell culture. The cells were collected by centrifugation, washed twice with phosphate buffered saline (PBS) buffer, and suspended in the PBS buffer to an appropriate cell density for green fluorescence assay on the microplate reader (BioTek Synergy H1N) with excitation/emission at 470 nm/510 nm. The EGFP fluorescence intensity measured by the microplate reader was expressed in arbitrary units as relative fluorescence units (RFUs). The PBS buffer was used to determine the background fluorescence intensity. EGFP production (RFU/OD) was calculated as the green fluorescence intensity by deducting the background intensity and then dividing by the OD_{600} value at each time point. A relative EGFP production rate was derived as the ratio of EGFP production to the incubation time (RFU/OD/min). An empirical mathematical model, the Hill equation (eq 1), was used to model EGFP production rate (y) as a function of the initial concentration of the synthetic homoserine lactones (x).

$$y = A + \frac{B[X]^n}{C^n + [X]^n} \quad (1)$$

Four parameters (A, B, C, n) were estimated to obtain the best fit curve by performing a nonlinear curve fitting using the experimental results (SigmaPlot 14, Systat Software, Inc., San Jose, CA). The parameter A is the baseline of EGFP production rate when the AHL initial concentration is 0 M. The parameter B is the maximum EGFP production rate. The parameter C is also called EC_{50}, which represents the AHL concentration that can induce a half-maximal EGFP production rate.

The EGFP production of the biosensor induced by native AHLs produced from *P. aeruginosa* or *B. thailandensis* was measured as noted below. Briefly, the biosensor cells harvested at exponential phase were added to the sterile filtrates from *P. aeruginosa* or *B. thailandensis*, and the resultant mixtures were incubated at 37 °C and 250 rpm. At each predetermined time interval, aliquots of the samples were withdrawn, and the cells were collected for EGFP fluorescence measurement by the microplate reader.

Quantification of Lycopene Production. Single colonies of biosensor cells were inoculated into 5 mL of LB broth with 100 μg/mL ampicillin and 50 μg/mL kanamycin and incubated overnight in a shaking incubator at 37 °C and 250 rpm. The biosensor cells were diluted 1×10^4 times and cultivated in fresh LB broth to an OD_{600} of 2.0 for induction with synthetic AHLs ($3OC_{12}$-HSL, C_{10}-HSL, or $3OHC_{10}$-HSL) or sterile filtrates from the bacterial culture. Aliquots of the sample were taken at different time points in the batch experiments, and the cells were collected for measuring lycopene production levels. To determine relative lycopene content, a quantitative absorbance-based method was adopted.[81,82] In this study, we determined the sensitive wavelength by obtaining the absorbance spectrum of lycopene in the engineered *E. coli* cells (Figure S8). A detailed description is provided in the Supporting Information. All measurements were conducted in triplicates in black 96-well plates with a clear bottom by a microplate reader (BioTek Synergy H1N).

The lycopene content [lycopene] in the cell suspension is proportional to the measured optical density of lycopene by subtracting the scattering component as follows

$$[\text{lycopene}] \propto \text{Abs}_{340\,\text{nm}} = \text{OD}_{340\,\text{nm}} - \text{OD}_{340\,\text{nm,scat}} \quad (2)$$

where $Abs_{340\ nm}$ represents the absorbance of lycopene at the sensitive wavelength of 340 nm and $OD_{340\ nm}$ is the measured optical density at 340 nm. $OD_{340\ nm,scat}$ is the scattering component contributed by the *E. coli* cell without lycopene at the wavelength 340 nm. The $OD_{340\,nm,scat}$ calculated in this study is based on the experimental measurement of the biosensor cells without induction by synthetic AHLs or filtered bacterial culture solutions. Lycopene production per cell was calculated by normalizing the lycopene content by OD_{600}.

Creation of the Paper-Based Assay. To assemble the paper-based assay, the biosensor cells were cultivated in LB medium at room temperature at 200 rpm to an OD_{600} of 2.0, and cell pellets were mixed with 2% alginate (Sigma, low viscosity) to give 10^9 cells per hydrogel spot on a qualitative filter paper (VWR, cat. 28130-026) with a diameter of ~5 mm. The filter paper with two different spots, a biosensor spot and a negative control spot (an *E. coli* cell with an engineered lycopene synthesis pathway in the reporting module but no QscR sensing module), was cut into small rectangles (20 mm × 10 mm). To characterize functionality, the small rectangular paper was dipped in 5 mL of solutions containing synthetic homoserine lactones $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL, respectively, at various concentrations of 1.0×10^{-6}, 1.0×10^{-7}, 5.0×10^{-8}, and 2.0×10^{-9} M. The lycopene readout for the paper-based assay was visually inspected and photographed. All experiments were performed in triplicates.

Fluorescence Microscope Imaging. The EGFP expressing cells were heat fixed on a glass slide and then visualized on an Eclipse 90i fluorescence microscope (Nikon Instruments Inc., Melville, NY). The green fluorescence signal was detected through a U-MWIB2 mirror unit with a BP460-490 excitation filter and a DM505 dichroic mirror with a BA510IF emission filter (Olympus). Images were analyzed with MetaMorph software (MDS, Inc., Sunnyvale, CA).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssynbio.0c00491.

Student's t test for the EFGP production rate of the biosensor induced by different AHLs (Table S1); plasmids and primers used in this study (Table S2); plasmid maps for pET23b-qscR and pUC57-egfp (Figure S1); successful expression of soluble QscR recombinant protein shown by Western blotting (Figure S2); response of the biosensor toward six synthetic AHLs (Figure S3); time series profiles of the biosensor EGFP production

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induced by $3OC_{12}$-HSL, C_{10}-HSL, and $3OHC_{10}$-HSL (Figure S4); EGFP production rate of the biosensors in tap water samples spiked with different concentrations of synthetic molecules $3OC_{12}$-HSL, C_{10}-HSL, or $3OHC_{10}$-HSL (Figure S5); plasmid maps of pET23b-qscR-EIB and pUC57-crtI (Figure S6); paper-based assays to detect AHL molecules in solution (Figure S7); optical density spectrum of the lycopene-producing biosensor and negative control strain and the spectrum of lycopene in *E. coli* cells calculated by the optical density of the lycopene-producing biosensor by subtracting the optical density of the negative control (Figure S8); determination of lycopene content by a microplate reader (PDF)

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Notes

The authors declare no competing financial interest.

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