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Paper Strip Whole Cell Biosensors: A Portable Test for the Semiquantitative Detection of Bacterial Quorum Signaling Molecules

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Herein, we report the development of a novel, inexpensive, and portable filter-paper-based strip biosensor for the detection of bacterial quorum sensing signaling molecules, *N*-acylhomoserine lactones (AHLs). AHLs are generally employed by Gram-negative bacteria for their cell−cell communication to control expression of specialized genes, such as those involved in biofilm formation and production of virulence factors, in a population-density-dependent manner. First, a bacterial cell-based sensing system employing components of AHL-mediated QS regulatory system as recognition elements and *β*-galactosidase as the reporter protein was designed and developed. The bacterial-sensing cells were then liquid-dried on strips of filter paper. *β*-Galactosidase as the reporter allows for the visual monitoring of the analyte-induced signal when a colorimetric method of detection is applied. The paper strip biosensor was able to detect low AHL concentrations down to 1×10^{-8} M. Furthermore, it was successfully applied to the detection of AHLs in physiological samples, such as saliva. The filter-paper-based sensing strips could provide reproducible results upon storage at 4 °C for at least 3 months. In conclusion, a filter-paper-based strip biosensor was developed that allows for visual, fast, and convenient detection of AHLs in a dose-dependent manner in a test sample. In addition, it does not require expensive equipment or trained personnel and allows ease of transportation and storage. Therefore, we envision that this biosensor will serve as a simple and economical portable field kit for on-site monitoring of AHL in a variety of clinical and environmental samples.

In the past two decades, quorum sensing (QS) has emerged as a key research area due to its involvement in various health and environmental issues.[1−4] It has become increasingly apparent that most bacteria responsible for infections and biofouling regulate their behavior by quorum sensing. Specifically, QS allows bacteria to coordinate their behavior in a group-based manner by controlling and synchronizing the expression of certain genes. This occurs thanks to the ability of bacteria to communicate among themselves by producing, releasing, and responding to certain chemical signaling molecules in a population-density-dependent manner. The most studied chemical signaling molecules involved in QS include *N*-acyl homoserine lactones (AHLs) in Gram-negative bacteria, a family of oligopeptides in Gram-positive bacteria, and autoinducer-2 (AI-2) in both Gram-positive and Gram-negative bacteria.[5] Several bacterial behaviors are regulated by QS: for instance, bioluminescence, swarming and motility, competence, sporulation, biofilm formation and production of virulence determinants. There are numerous reports in the literature that support the involvement of QS in the pathogenesis of bacteria-related disorders.[6−12] In addition, it has been reported that about 80% of microbial infections involve biofilm formation.[13] Quorum-sensing molecules (QSMs) have also been demonstrated to be associated with environmental concerns such as biofouling.[14−16]

Consequently, it is very important to understand bacterial communication. Toward this goal, there is a need for methods proficient at detecting and quantifying QSMs in biological and environmental samples in a sensitive and selective manner. Various analytical methods have been developed for the detection of AHLs in the supernatants of bacterial cultures. Such methods are based on either bacterial whole-cell sensing systems or

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conventional physical-chemical techniques, including colorimetry as well as liquid and gas chromatography with UV, mass spectrometry, or diode array detection, among others.[17−23] Only a few of these analytical methods have been applied to AHL monitoring in biological and physiological samples; a comprehensive discussion can be found in a review by Kumari et al.[24] Previously, we have developed and employed both a LC/MS/MS method[24] and genetically engineered bacterial whole-cell sensing systems[25] for the detection of AHLs in biological samples, such as saliva and stool. We have demonstrated that the bacterial sensing systems, based on bioluminescent *luxCDABE* reporter gene constructs, allow rapid, sensitive, cost-effective, and quantitative detection of AHLs.[25]

The next step in our research is the incorporation of whole-cell sensing systems into self-contained sensing devices that could routinely be employed for on-site monitoring of QSMs in biological and environmental samples. Whole cell biosensing systems have several features that render them suitable components of portable field kits. These characteristics include ability to withstand environmental conditions, such as a wide ranges of temperatures, pHs, and ionic strengths; provide information on the analyte bioavailability; requirement of minimal or no sample pretreatment; high sensitivity and selectivity; ease of preparation; easy and rapid detection; cost-effectiveness; amenability to high-throughput screening; miniaturization; and automation.[26,27]

Genetically engineered whole cells have been integrated into various solid supports to achieve portable biosensors. Toward this objective, gold or silica surfaces, optical fibers, microtiter plates, microfluidic platforms, microarrays, integrated circuit chips, and paper strips have been employed.[28] A variety of matrixes such as agar medium, agarose, alginates, sol−gel-derived silicates, dialysis membranes, and drying protectant solutions have been utilized to immobilize recombinant whole cells.[29−31] Despite the availability of these multiple approaches, there is a paucity of reports of integrated analytical devices capable of on-site monitoring of AHLs. For example, the production or inhibition of purple color in *Chromobacterium violaceum* has been employed for AHL sensing purposes.[32−34] Even though it allows for visual monitoring of AHLs without requiring instrumentation, the detection is not in a dose-dependent manner. Recently, Yong and Zhong developed a blue-green pigment-based genetically engineered whole cell biosensor for the detection of *N*-butyryl homoserine lactone. However, it should be noted that both of these methods are tedious, costly, and time-consuming (at least 16−18 and 24 h, respectively), thus not being suitable as portable field kits.

In this work, we report the development of a self-contained sensing system for the detection of AHLs that is based on genetically engineered bacterial cells that are directly dried on filter paper strips. These bacterial sensing cells employ β-galactosidase as the reporter protein, which can visually be detected by using an appropriate chromogenic enzyme substrate. The sensing system was validated by application to the detection of AHLs in human saliva. The paper strip biosensor allowed for visual, fast, convenient, and dose-dependent monitoring of AHLs in a test sample, thus proving to be a valuable portable tool for on-site analysis.

EXPERIMENTAL SECTION

Materials. *N*-Dodecanoyl-DL-homoserine lactone (C12-HSL), Whatman general purpose filter paper (medium speed, creped, diameter 9.0 cm), peptone from animal tissue, meat extract, gelatin from porcine skin, sodium L-ascorbate, D-(+)-raffinose pentahydrate, L-glutamic acid monosodium salt monohydrate, ampicillin, and anhydrous *N*,*N*-dimethylformamide (DMF) were purchased from Sigma (St. Louis, MO). Acetonitrile was of HPLC grade from Fisher Scientific (Pittsburgh, PA). HEPES, sodium chloride, potassium chloride, magnesium chloride, sodium phosphate monohydrate, and disodium phosphate heptahydrate were also from Fisher Scientific (Pittsburgh, PA). Luria−Bertani (LB) agar and broth were purchased from Difco (Sparks, MD). DNA primers were purchased from Operon Biotechnologies (Huntsville, AL). The chromogenic substrate X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) was from Gold BioTechnolgy (St. Louis, MO). The chemiluminescence detection kit, Beta-Glo Assay System, was from Promega (Madison, WI). The 96-well microtiter plates were purchased from Costar (Corning, NY). The microcentrifuge was from Eppendorf (Westbury, NY).

The alpha 2-4 lyophilizer was from Martin Christ Gefriertrocknungsanlangen GmbH (Osterode, Germany). The orbital shaker incubator was from Fisher Scientific (Fair Lawn, NJ). Chemiluminescent measurements were performed using the FLUOstar OPTIMA microplate reader (BMG Labtech, Durham, NC). A Pentax K20D digital camera (Pentax, Golden, CO) was used for taking jpeg images of the strips.

Plasmids, Bacterial Strains and Culture Conditions. Competent *Escherichia coli* DH5α-T1 cells were purchased from Invitrogen (Carlsbad, CA). The plasmid pSB1075 was provided by Dr. Paul Williams (University of Nottingham, Nottingham,

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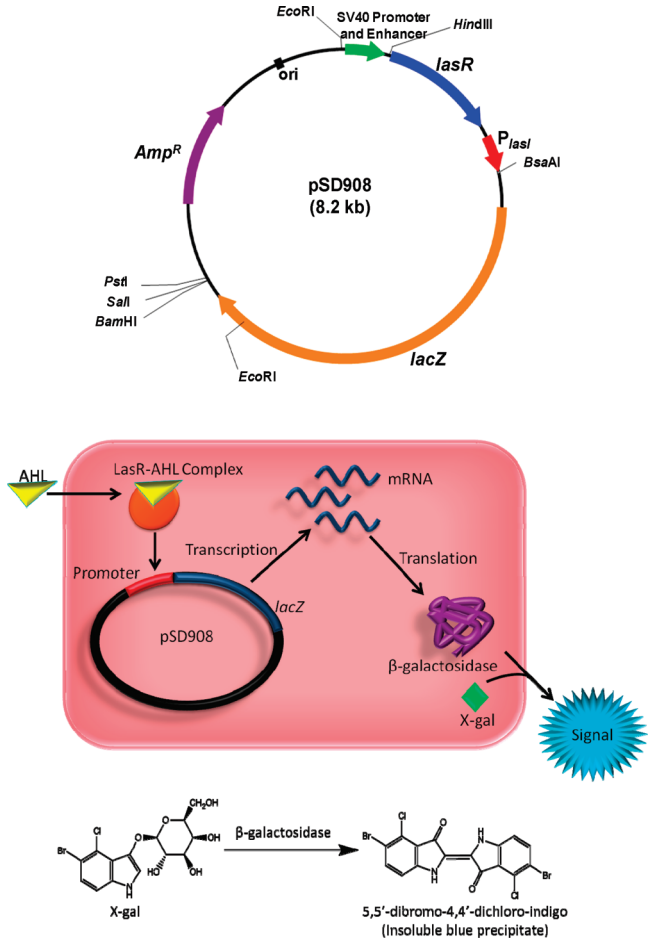

Figure 1. Schematic of the plasmid pSD908 constructed for AHL sensing (top). In the presence of AHL, the regulatory protein LasR forms a regulatory protein−AHL complex. The resulting LasR−AHL complex then binds to its corresponding promoter (P_{lasI}) and activates transcription of the *lacZ* reporter gene, resulting in the expression of the reporter protein, β-galactosidase. The amount of the reporter protein produced can be measured using a colorimetric-based method of detection, where X-gal is cleaved by β-galactosidase to form an insoluble blue product (bottom).

U.K.). The plasmid vector pSV-β-galactosidase was purchased from Promega (Madison, WI).

The gene sequences corresponding to P_{lasI} and *lasR* from the *lasR*/*lasI* AHL-mediated QS regulatory system of *Pseudomonas aeruginosa* were isolated from plasmid pSB1075[35] using polymerase chain reaction (PCR). The plasmid pSD908 (Figure 1, top) was prepared by inserting a *Hin*dIII−*Bsa*AI fragment containing the P_{lasI} and *lasR* sequences from the plasmid pSB1075 between the *Hin*dIII−*Bsa*AI restriction sites of plasmid vector pSV-β-galactosidase. The resulting pSD908 vector contains the *lasR* gene, and the *lacZ* gene, encoding β-galactosidase, which is under the transcriptional control of P_{lasI}. The construction of pSD908 was verified by digesting the plasmid with *Hin*dIII and *Bsa*AI and confirming the lengths of the digested fragments on a 1% agarose gel. The plasmid pSD908 was transformed into competent *E. coli* DH5α-T1 cells using the standard protocol provided by the manufacturer. The transformed cells were grown at 37 °C overnight on LB agar plates containing 100 μg/mL ampicillin. Cells from a single colony were grown overnight at 37 °C, with shaking at 250 rpm in LB broth containing the same amount (w/v) of ampicillin. Finally, glycerol stocks were prepared from these cell cultures and stored at −80 °C. Fresh cell cultures were obtained from the glycerol stocks and grown at 37 °C, with shaking at 250 rpm in LB broth, containing 100 μg/mL ampicillin, until the desired optical density at 600 nm (OD_{600nm}) was reached.

Sample Collection and Preparation. Saliva samples were employed for the detection of AHLs. The samples were obtained from patients with gastrointestinal (GI) disorders and healthy volunteers. Sample collection and preparation was performed as described earlier. Briefly, saliva samples were collected in the mornings after brushing teeth to minimize bulk collection of debris and the presence of oral bacteria. The samples were then processed by simple centrifugation in sterile Eppendorf tubes at 13 000 rpm for 2−3 min at room temperature. Supernatants were then stored at −80 °C until analyzed.

Dose−Response Curves in Microtiter Plates. Fresh cell cultures at an OD_{600nm} of 0.45−0.50 were obtained from the glycerol stocks as described above. Commercially available C12-HSL was dissolved in acetonitrile to obtain 1×10^{-2} M stock solution, which was serially diluted with either reverse osmosis (RO) filtered water (Milli-Q Water Purification System, Millipore, Bedford, MA) or pooled saliva. AHL standard solutions of concentrations ranging from 1×10^{-4} M to 1×10^{-9} M were obtained. A 1% solution of acetonitrile in RO filtered water and pooled saliva, respectively, was used as the blank. A 10-μL volume of each of the AHL solutions was added in triplicate to the wells of a 96-well microtiter plate containing 90 μL/well of cell culture. The microtiter plate was incubated in an orbital shaker at 37 °C at 150 rpm for 1.5 h, and then equilibrated to room temperature for 10 min. The amount of reporter protein β-galactosidase expressed was determined using the chemiluminescence-based detection kit, Beta-Glo Assay System, following the manufacturer's instructions. The induced chemiluminescence was measured (1s/well), and the light intensity was expressed in relative light units (RLU). The results were plotted using the software GraphPad Prism 4 (GraphPad Software, Inc., San Diego, CA).

Preparation of Filter-Paper-Based Sensing Strips. Paper sensing strips were prepared as described by van der Meer and co-workers with modifications.[36] Briefly, cells were suspended in sterile preheated (37 °C) drying protectant solution.[36,37] A volume of 3 μL of this sensing cell suspension was spotted on Whatmann filter paper strips (0.6 × 4 cm), which were then dried for 10 min in a laminar air flow cabinet and subsequently dried in vacuum at 20 °C in a lyophilizer. The filter paper sensing strips prepared were then stored at 4 °C until used.

Dose−Response Curves on Filter-Paper Strips. Commercially available C12-HSL was dissolved in acetonitrile to obtain 1×10^{-2} M stock solution, which was serially diluted with 50 mM HEPES buffer (pH 7.0) containing 50 mM each of sodium chloride, potassium chloride, and magnesium chloride. HEPES

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buffer containing these salts was used for all the filter-paper-strip-based experiments. AHL standard solutions of concentrations ranging from 1×10^{-4} M to 1×10^{-9} M were obtained. A 1% solution of acetonitrile in buffer was used as the blank. A volume of 100 μL of each of these standard solutions was added to the culture tubes containing 900 μL of LB broth with 100 μg/mL ampicillin. The LB broth was prepared in tap water and then autoclaved. Previously prepared filter-paper strips were inserted in these culture tubes, which were then incubated either at 37 °C or room temperature (RT) without shaking for 1.5 h. The strips were then taken out of the culture tubes and kept between layers of plastic wrap to prevent drying. Subsequently, 10 μL of X-gal substrate solution (50 mg/mL) in DMF was added carefully on the spot with sensing cells. Color development was carried out for 90 min. In addition to visual observation of the blue color developed, the color intensities were measured using the software ImageJ (NIH, Bethesda, MD) (http://rsbweb.nih.gov/ij/) upon digital image acquisition. Briefly, the measurement settings in ImageJ were set to mean gray value and the images were inverted. ImageJ records 100% black as zero and 100% white as 255; thus, inversion of the images was necessary. A rectangle section was drawn around a spot to be measured using selection tool. The size of the rectangle drawn was maintained at 4×8 mm^2 area, and the mean gray value was recorded. A background measurement was taken for the same sized rectangle box area closest to the spot on the strip, which accounted for variations in color intensity due to differences in environmental illumination while the pictures were taken. Essentially, the final color intensity signals were corrected with respect to both the blank and illumination-related variations in color intensity.

Analysis of Saliva Samples on Filter Paper Strips. Saliva samples were collected and processed as described above. A volume of 100 μL of each of the processed saliva samples was added in triplicate to culture tubes containing 100 μL of HEPES buffer and 800 μL of LB broth, and the assay was performed as described above. A dose−response curve using standard AHL solutions was also obtained as a reference in each analytical run. The blue color developed for saliva samples was then compared with the spots on the calibration strips.

RESULTS AND DISCUSSION

The contribution of quorum sensing to various health and environmental issues has largely been demonstrated in recent years. Therefore, it is crucial to elucidate the mechanisms involved in bacterial communication and the role that QS plays in these hazards. For that, novel methods capable of detecting bacterial chemical signaling molecules at low levels, on-site, and in real samples are needed. Specifically, the availability of portable sensing systems to detect QSMs would facilitate on-field environmental studies as well as clinical studies in the physician's office and at the patient's bedside. We previously showed that genetically engineered bioluminescent bacterial whole-cell sensing systems enable sensitive, selective, rapid and quantitative detection of AHLs in physiological matrices, such as stool and saliva.[24,25] The aim of the present work was to design and construct new recombinant sensing cells suitable for visual signal detection and utilize them in the implementation of a novel, convenient, and self-contained portable sensor for on-site detection of QSMs. To that end, we developed a filter-paper-based strip sensor in which the genetically engineered cells were directly dried on filter paper strips. Recently, a significant amount of work has been performed by several researchers in the development of paper-based analytical devices incorporating biological agents.[36,38−41] The design, preparation, characterization, and immobilization of the sensing cells, as well as the analytical characterization and validation in saliva samples of the paper strip biosensor are discussed below.

In whole-cell sensing systems, the host cell usually contains a plasmid that is genetically engineered by coupling a sensing element with a reporter gene. In inducible-operon-based whole-cell sensing systems, the sensing element in the plasmid contains an operator/promoter (O/P) region and a regulatory gene that encodes for a recognition/regulatory protein. The expression of the reporter gene is under the transcriptional control of the O/P. When the target analyte is present in a test sample, a regulatory protein−analyte complex is formed. In positive regulation sensing systems, the resulting complex then binds to the promoter, activating expression of genes that are under the transcriptional control of the promoter, including the reporter gene. The expression of the reporter protein produces a measurable signal that can be correlated with the concentration of analyte present.

In this work, a genetically engineered *E. coli* sensing system, with β-galactosidase as the reporter protein, was designed and developed. The newly constructed plasmid vector pSD908 is based on the *lasR*/*lasI* QS regulatory system of *P. aeruginosa*.[35] It contains the *lasR* regulatory gene, P_{lasI} promoter sequence, and *lacZ* gene, which is under the transcriptional control of P_{lasI} (Figure 1, bottom). The *lasR* encodes for LasR regulatory protein as the recognition element, which is known to respond to long-acyl-chain AHLs (C8 and above).[25] The *lacZ* encodes for β-galactosidase, a hydrolase enzyme, which has been extensively employed as a reporter. A variety of substrates are available for measuring its enzymatic activity. Depending on the type of substrate chosen, the signal produced can be detected using colorimetric, electrochemical, fluorescent, or chemiluminescent methods.[42−44] When AHLs are present in the environment of the whole sensing cells, the recognition/regulatory protein LasR forms a LasR−AHL complex. The resulting complex then binds to its corresponding promoter P_{lasI} and activates transcription of the *lacZ* reporter gene. Upon addition of the chosen enzyme substrate, a corresponding signal is produced due to the enzymatic activity of β-galactosidase. This signal is directly proportional to the amount of the AHL molecule present in the environment of the sensing cells.

To evaluate the analytical performance of the developed whole cell sensing system, we incubated suspensions of the *E. coli* cells containing the plasmid pSD908 with varying concentrations of C12-

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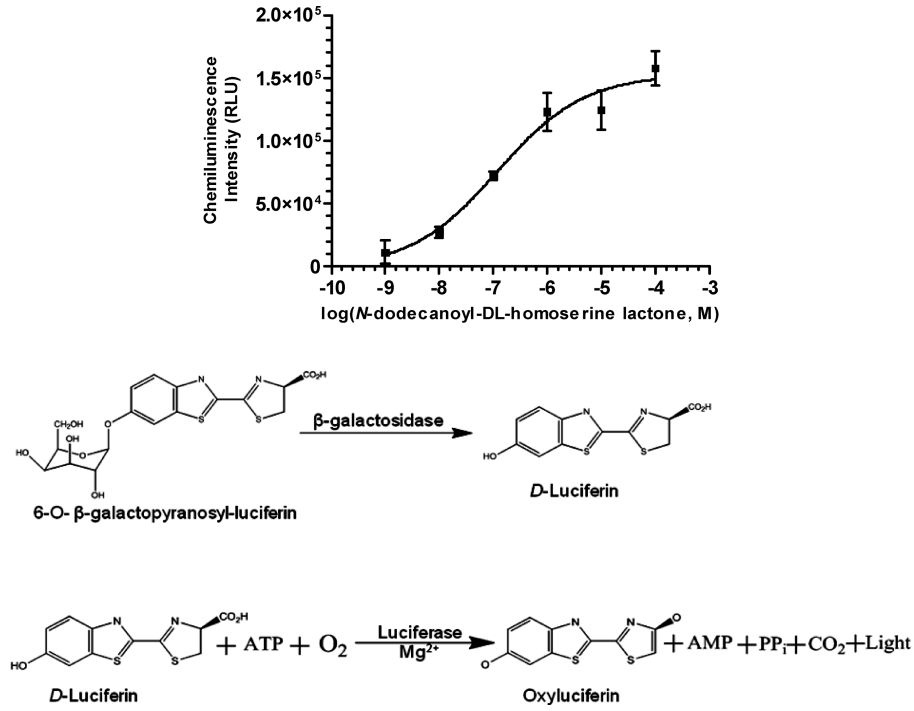

Figure 2. Calibration plot for the biosensing system employing plasmid pSD908. Bacterial cells were incubated with various concentrations of *N*-dodecanoyl-DL-homoserine lactone in a microtiter plate for 1.5 h at 37 °C with continuous shaking. The chemiluminescent signals have been corrected with respect to the blank. Data shown are the average ± 1 SD ($n = 3$) (top). The coupled enzyme reactions were catalyzed by β-galactosidase and luciferase, respectively, which produce the signal (bottom). Specifically, β-galactosidase cleaves 6-*O*-β-galactopyranosyl luciferin to yield luciferin, which serves as the substrate for luciferase to yield a light signal in the presence of cofactors. The amount of reporter protein β-galactosidase expressed (corresponding to the analyte concentration) correlates with the amount of luminescence produced and measured.

HSL (1×10^{-4} M to 1×10^{-9} M), which was previously demonstrated to induce high response, with the lowest limit of detection, in a whole cell sensing system also based on *lasR*/P_{lasI} as the recognition element.[25] The expression of β-galactosidase was then monitored using a chemiluminescence-based detection system, as shown in Figure 2 (bottom). An increase in chemiluminescence intensity was observed with increasing concentrations of C12-HSL over a concentration range of 4 orders of magnitude (Figure 2, top). The limit of detection was defined as the analyte concentration producing a signal equal to or higher than the average signal produced by the blank plus three standard deviations and resulted to be 1×10^{-9} M. The whole cell sensing system proved to be precise, with within-assay coefficients of variation less than 5%.

Although we employed X-gal based colorimetric detection for filter-paper assays, the same chromogenic substrate could not be applied to the optimization experiments performed with suspension cells. The reason for this is the formation of a colored precipitate upon X-gal addition to β-galactosidase, which is required for colorimetric/visual detection when the sensing cells are immobilized on a solid support. We also assessed whether the sample matrix affected the detection of AHLs in saliva using our whole cell sensing system. It should be noted that we have previously demonstrated the presence of AHLs in saliva from both healthy volunteers and individuals with GI disorders.[24,25] Additionally, the use of saliva as the test specimen presents the advantage of enabling noninvasive collection of samples. To that end, we added known concentrations of C12-HSL to pooled saliva, previously determined not to contain AHLs, and obtained a dose−response curve. Upon comparison with a reference dose−response curve obtained in the absence of saliva during the same analytical run, overlapping curves, with nearly identical slopes were observed, thus showing no significant saliva matrix effect (Figure 3).

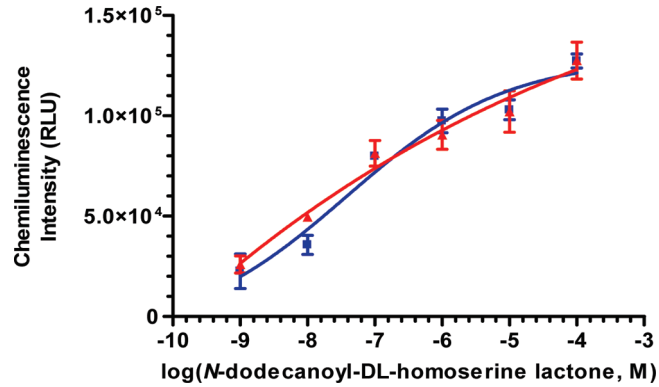

Figure 3. Evaluation of the saliva matrix effect using the biosensing system containing plasmid pSD908. Dose−response curve achieved by analyte standard additions to a pooled saliva sample (red triangle) and reference calibration plot (blue filled box) obtained in a microtiter plate during the same analytical run. The chemiluminescent signals have been corrected with respect to the blank. Data shown is the average ± 1 SD ($n = 3$).

After evaluating the analytical performance of the whole cell sensing system in suspension experiments, we immobilized the genetically engineered *E. coli* cells containing the plasmid pSD908 to prepare a filter-paper-based strip sensor for the detection of AHLs. For that, we followed the approach suggested by Stocker

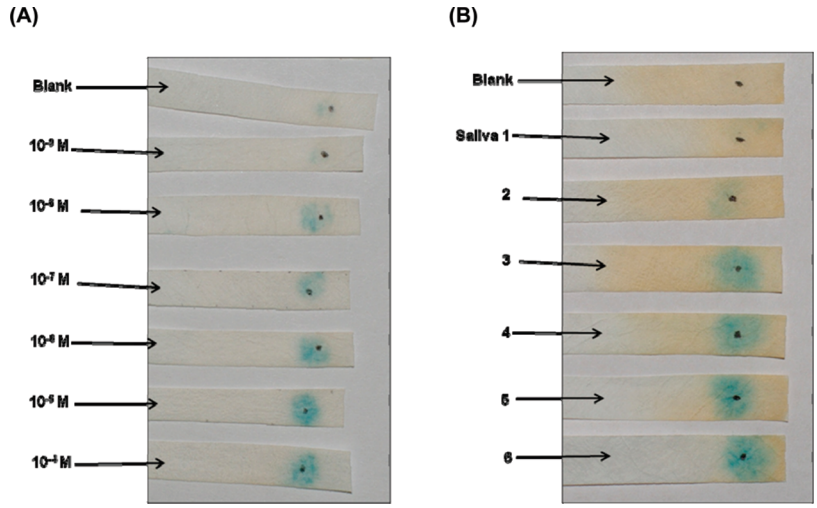

Figure 4. (A) Dose−response data for the biosensing system employing plasmid pSD908 on the filter-paper-based strips. Filter paper strips were incubated with various concentrations of *N*-dodecanoyl-DL-homoserine lactone and LB broth (containing 100 μg/mL ampicillin) for 1.5 h at 37 °C without shaking. Acetonitrile, 1%, in HEPES buffer served as the blank. Subsequently, the strips were taken out and kept on plastic wrap and then 10 μL of X-gal substrate solution (50 mg/mL) in DMF was added directly on the spots with sensing cells. The plastic wrap was then closed to prevent drying, and the color development was carried out for 90 min. (B) Detection of AHLs in saliva samples using the filter-paper-based strip biosensor containing the plasmid pSD908. Samples 1−5 were from five healthy volunteers and sample 6 was from one patient with Crohn's disease.

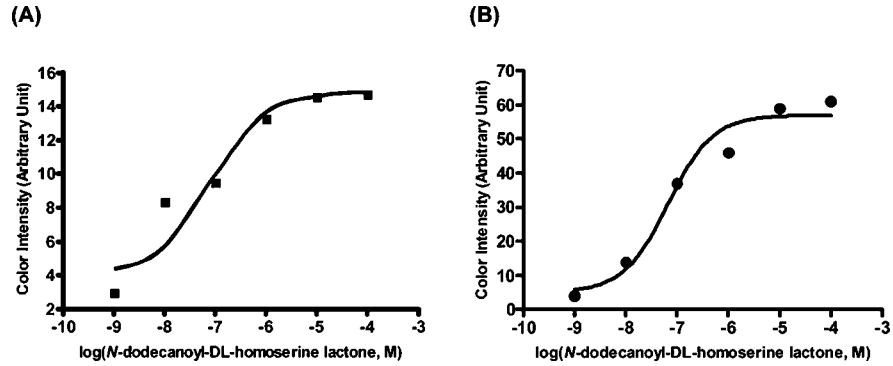

Figure 5. (A) The intensity of blue color developed on the spots of the calibration strips obtained by incubation with the analyte at 37 °C (Figure 4A) was measured using the software ImageJ upon digital image acquisition. (B) Similarly, the intensity of blue color developed was measured on the spots of the calibration strips obtained by incubation with the analyte at RT. The signals have been corrected with respect to the blank and variations in color intensity due to differences in environmental illumination while the pictures were taken.

et al.[36] with experimental modifications. Specifically, the developed bacterial sensing cells were suspended in a drying protectant solution and directly dried on a filter-paper strip under controlled conditions, as described in the Experimental Section. The strips were dried using a liquid-drying method, which allows for vacuum-drying of samples from the liquid state without harsh freezing conditions.[37] This mild drying method is expected to help in long-term preservation of cells. The modified paper strip was immersed in a test solution in the presence of LB broth at 37 °C for 1.5 h, followed by the addition of X-gal onto the bacterial cells for the development of a blue color. An increase in blue color intensity was observed with increasing concentrations of C12-HSL (Figure 4 A). The visual results were confirmed by measuring the color intensities with appropriate software, as shown in Figure 5 A. The paper strip biosensor was able to detect low AHL concentrations down to 1×10^{-8} M upon color development for 90 min. However, a dose-dependent response with a limit of detection of 1×10^{-7} M was observed after 60 min incubation with the chromogenic substrate. Since the paper strips can visually be monitored over time, the presence of higher AHL concentrations in samples can be determined at an earlier time.

Figure 6 shows the results obtained across three individual experiments. Furthermore, the assay was performed by incubating the sensing strips with test solutions at RT. A dose-dependent increase in blue color intensity was also observed. Measurement of color intensities proved the visual results and showed a limit of detection of 1×10^{-8} M (Figure 5B), as in the corresponding assay carried out at 37 °C. These results demonstrate the applicability of the developed paper-based sensing system, not only in point-of-care testing, but also in field analysis in remote locations with limited facilities. This protocol allowed for the fast and convenient visual monitoring of AHLs in a dose-dependent manner. Moreover, the strips could be stored for 3 months at 4 °C without a noticeable decrease in the sensing cells' ability to quantitatively respond to the analyte.

The paper strip biosensor was also validated by employing it for the detection of AHLs in saliva. Distinct levels of AHLs were

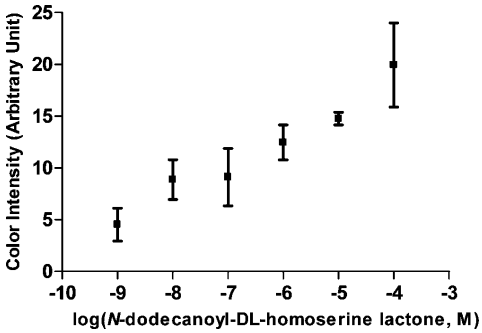

Figure 6. The intensity of blue color developed on the spots of the calibration strips obtained in the three individual assays by incubation with the analyte at 37 °C was measured using the software ImageJ upon digital image acquisition. The signals have been corrected with respect to the blank and variations in color intensity due to differences in environmental illumination while the pictures were taken. Data shown is the average ±1 SD ($n = 3$).

detected in saliva of healthy and diseased individuals (Figure 4B). Overall, the results obtained demonstrate that filter-paper-based sensor strips can successfully be used for AHL detection in physiological samples such as saliva.

CONCLUSIONS

Herein, we have developed a self-contained portable sensing system based on genetically engineered whole cells physically adsorbed on filter-paper strips for on-site semiquantitative visual monitoring of AHLs in a test sample. Additionally, quantitative measurements could be performed by means of digital image analysis. This novel filter-paper strip biosensor provides a fast and convenient method for the detection of AHLs, which could be employed for first-level screening of a variety of environmentally and clinically relevant samples. The sensing system could also be utilized for evaluating the presence of molecules able to interfere with QS, including agonists and antagonists. A filter-paper-based strip sensor is amenable to high-throughput analysis, provides easy transportation and storage, and does not require instrumentation or trained personnel; therefore, it could be a component of a simple and inexpensive field kit.

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