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Whole-Cell Biosensors as Tools for the Detection of Quorum-Sensing Molecules: Uses in Diagnostics and the Investigation of the Quorum-Sensing Mechanism

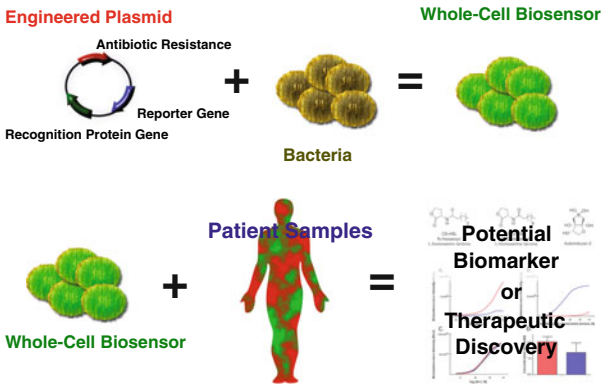
Gregory O'Connor, Leslie D. Knecht, Nelson Salgado, Sebastian Strobel, Patrizia Pasini and Sylvia Daunert

Abstract Genetically engineered bacterial whole-cell biosensors are powerful tools that take advantage of bacterial proteins and pathways to allow for detection of a specific analyte. These biosensors have been employed for a broad range of applications, including the detection of bacterial quorum-sensing molecules (QSMs). Bacterial QSMs are the small molecules bacteria use for population density-dependent communication, a process referred to as quorum sensing (QS). Various research groups have investigated the presence of QSMs, including *N*-acyl homoserine lactones (AHLs) and autoinducer-2 (AI-2), in physiological samples in attempts to enhance our knowledge of the role of bacteria and QS in disease states. Continued studies in these fields may allow for improved patient care and therapeutics based upon QSMs. Furthermore, bacterial whole-cell biosensors have elucidated the roles of some antibiotics as QS agonists and antagonists.

G. O'Connor · L.D. Knecht (✉) · N. Salgado · S. Strobel · P. Pasini · S. Daunert
Department of Biochemistry and Molecular Biology, University of Miami, Miller School of Medicine, Miami, FL 33136, USA
e-mail: lknecht@miami.edu

L.D. Knecht
Department of Chemistry, University of Miami, Miami, FL 33146, USA

Graphical Abstract



Keywords Autoinducer-2 (AI-2) · Cystic Fibrosis (CF) · Inflammatory Bowel Disease (IBD) · *N*-acyl homoserine lactones (AHLs) · Quorum-sensing (QS) · Quorum-sensing molecules (QSMs) · Urinary Tract Infections (UTI) · Whole-cell biosensors

Contents

1 Introduction

Quorum sensing (QS) is the population-dependent exchange of small chemical signaling molecules that allow bacteria to monitor their population density and regulate gene expression accordingly. The phenomenon was first described in the early 1970s with the microorganism *Vibrio harveyi*, which employs QS to control the emission of light (bioluminescence) [1, 2]. Traditionally, these signaling molecules have been classified into two subgroups: *N*-acyl homoserine lactones (AHLs, Fig. 1), which are used by Gram-negative bacteria, and oligopeptides,

which are used by Gram-positive bacteria. In recent years, however, it has been found that quorum-sensing molecules (QSMs) are not limited to only these two subgroups, with the discovery of pseudomonas quinolone signal (PQS), autoinducer 2 (AI-2, Fig. 1), and autoinducer 3 (AI-3), among others; interestingly, some of these molecules, such as AI-2, can appear in both Gram-positive and Gram-negative bacteria [3, 4]. QSMs are estimated to influence 4–10 % of the bacterial genome and 20 % of the proteome, regulating myriad genes ranging from factors responsible for bacterial virulence to genes involved in basic metabolic processes [5]. For example, QSMs allow bacteria to communicate with each other to form biofilms, an extracellular matrix system that helps protect the bacteria: most bacteria reside in these three-dimensional matrix systems rather than existing in the planktonic form [6]. In order for bacteria to be able to respond to QSMs, quorum-sensing systems comprise recognition/regulatory proteins that are able to bind to the QSMs and regulate gene expression, as well as synthases which allow for production of QSMs. Table 1 provides examples of quorum-sensing organisms, their regulatory proteins, and QSMs.

Previously, studies on QS and the mechanism of action of the QSMs mostly relied on the native bacteria and examining growth rates and up- or down-regulation of specific genes. Although these methods would give qualitative information about the effects of QSMs on the microorganisms, the amount of quantitative information was limited. With the identification of QS regulatory proteins and their promoter binding regions, genetically engineered whole-cell bacterial biosensors (also called

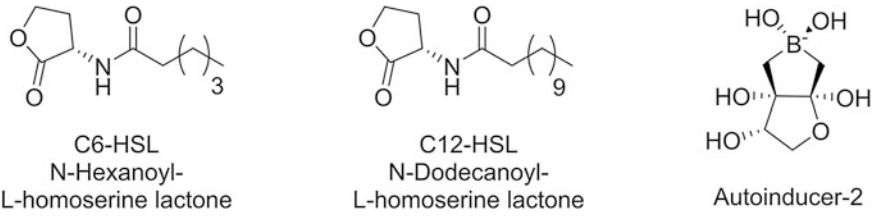


Fig. 1 Chemical structures of *N*-hexanoyl-*L*-homoserine lactone, *N*-dodecanoyl-*L*-homoserine lactone, and autoinducer-2 (boron form) QSMs

Table 1 Organisms and their QSMs that have been utilized in whole-cell biosensors

Organism	QSM	Synthase	Receptor/regulator
Pseudomonas aeruginosa	C4-HSL	RhlI	RhlR
	3-OXO-C12-HSL	LasI	LasR, QscR
	HHQ, PQS	PqsA	PqsR
Vibrio fischeri	3-oxo-C6-HSL	LuxI	LuxR
	AI-2	AinS	AinR
	CAI-1	LuxS	LuxP
Agrobacterium tumefaciens	3-oxo-C8-HSL	TraI	TraR

biosensing systems) have emerged as tools for detection of QSMs and understanding of the quorum-sensing mechanism [7]. These biosensing systems generate a detectable response under certain conditions, often times dose-dependent upon recognition of a specific analyte of interest. Whole-cell bacterial biosensors employ bacteria that have been genetically modified to contain a biological recognition agent coupled with a reporter. This genetic modification generally occurs in two ways: insertion of a genetically engineered plasmid and/or modification of the bacterial genome.

Developing a plasmid for use in a biosensing system allows for customization of a sensor depending upon the need, thus there is a large degree of variability. Several key elements that are required and/or commonly employed include genes for: a reporter protein, promoter region, antibiotic resistance, and over expression of the regulatory protein. The reporter plays a large role in the sensitivity of the biosensor, because it is the recognition agent for the assay. The reporter protein(s) selected for bacterial whole-cell biosensors are generally ones that can be detected optically, via an instrument or the naked eye (bioluminescence, chemiluminescence, fluorescence, and colorimetry), or electrochemically. Table 2 contains a list of a few common reporters, along with their detection methods and some advantages and disadvantages. The promoter region determines the production of the reporter protein, as well as playing a role in the specificity and selectivity of the system, thus it should be selected based upon the analyte of interest for the sensor. Including antibiotic resistance under a constitutive promoter allows for easy selection of bacteria expressing the plasmid, thus preventing the loss of plasmid as the bacterial culture grows. Overexpression of a regulatory protein increases the sensor's ability

Table 2 Commonly used reporter proteins for bacterial whole-cell-based sensing systems

Gene	Protein	Detection Method	Advantages	Disadvantages
lux	Bacterial luciferase	Bioluminescence	Ease of measurement, rapid response	Heat lability, oxygen requirement
luc	Firefly luciferase	Bioluminescence	High sensitivity, no heat lability	Exogenous substrate requirement, oxygen requirement
gfp	Green fluorescent protein (jellyfish)	Fluorescence	No substrate requirement	Low sensitivity, broad absorption spectra
lacZ	β-Galactosidase (*E. coli*)	Chemiluminescence, bioluminescence, fluorescence, colorimetry, electrochemistry	Wide variety of detection methods, detection by naked eye	Exogenous substrate requirement

Adapted from Raut et al. [7]

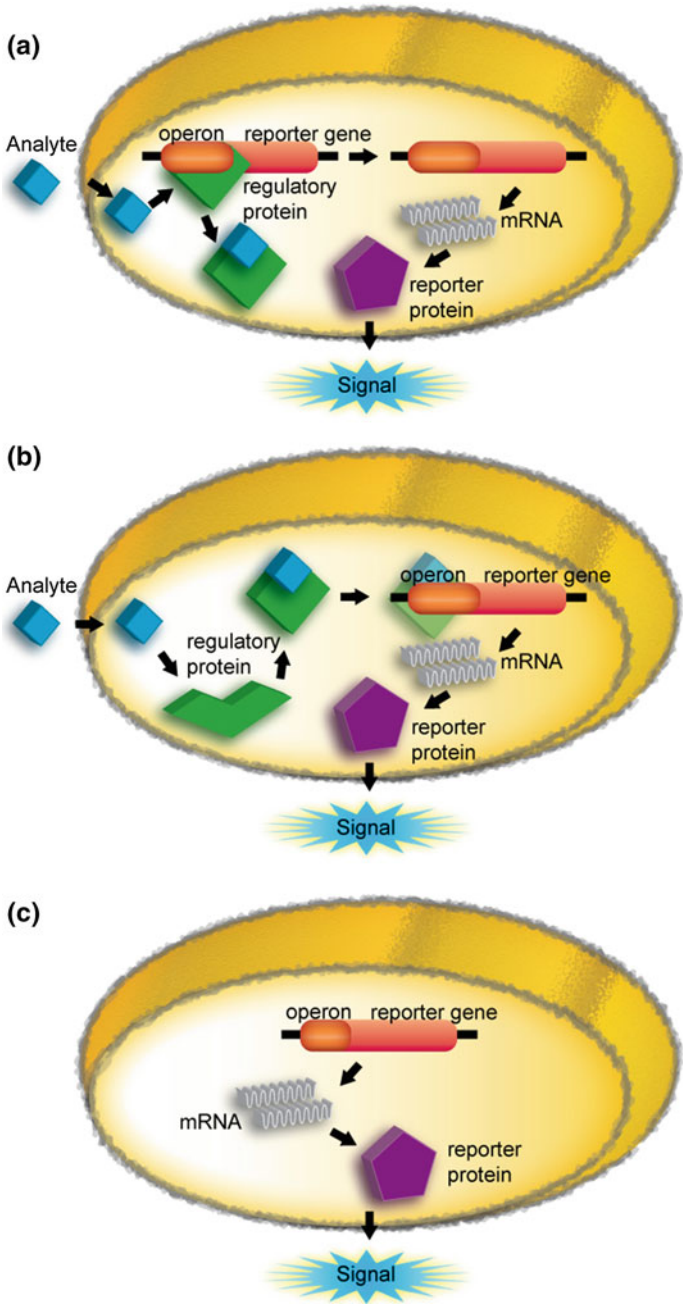
to detect the analyte of interest: the more protein you have, the higher its chances of encountering the analyte. Once a plasmid has been genetically engineered, it is a relatively simple task to insert the DNA into electro- or chemically competent bacterial cells, thus creating a whole-cell bacterial biosensor.

In addition to genetically engineering a plasmid to employ in a whole-cell biosensor, it is possible to modify the bacterial genome to exploit the original organism itself as a whole-cell biosensor. Many bacterial strains generate QSMs through various synthesis pathways, thus modifications can be made to bacterial genomes to prevent the generation of and/or the response to QSMs. One such example is the *V. harveyi* strain BB170; native *V. harveyi* activates its bioluminescence in response to AHLs and AI-2 and can produce these QSMs. The BB170 strain was genetically modified to have its AHL detection pathway, as well as its ability to synthesize AHLs and AI-2, blocked. Thus BB170 was successfully engineered to only generate bioluminescence in response to exogenous AI-2, allowing it to function as a biosensing system.

Regardless of the method used to develop the sensing system, bacterial whole-cell biosensors are categorized into two general types based upon whether expression of the reporter protein is inducible or constitutive. Inducible expression systems employ cells that have been genetically modified to contain an inducible promoter fused to a reporter gene. In the absence of the analyte (inducer), the reporter gene is expressed at low basal levels, determining the "blank" level of the system. When the analyte (inducer) is present, the reporter gene is expressed in a dose-dependent manner within a certain range of concentrations, allowing for a standard curve to serve as a control for sample measurements. Reporter gene expression can be negatively or positively regulated, defined by how the analyte–protein complex interacts with the gene promoter. In negative regulation (Fig. 2a), the regulatory protein is bound to the operator/promoter region, thus preventing reporter and other downstream gene expression. The analyte can bind the regulatory protein, causing the complex to be released from the promoter region and allowing for gene expression to occur. In positive regulation (Fig. 2b), the unbound regulatory protein complexes with the analyte and this complex can then bind to the operator/promoter region to activate reporter gene expression.

In contrast to inducible expression, constitutive expression systems are based upon generation of high basal levels of reporter expression (Fig. 2c). This approach is primarily employed in bacterial whole-cell biosensors as a measure of toxicity: however, nonspecific increased toxicity leads to cell death, reducing the amount of reporter protein and thus the overall signal. Therefore overall toxicity can be recorded, although it does not provide specific information about the nature and mode of action of the toxic agent(s) that is (are) killing the cells.

Bacterial whole-cell biosensors possess several advantages that have increased their popularity in recent years: they are relatively easy and inexpensive to prepare and store, are robust with a tendency to be stable to environmental changes such as pH or temperature fluctuations, can be engineered for multiplexing by using different recognition–reporter pairs, and can be integrated into various platforms allowing for high-throughput or on-site detection. In addition, regulatory proteins


(a)
Analyte
operon
reporter gene
regulatory protein
mRNA
reporter protein
Signal
(b)
Analyte
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mRNA
reporter protein
Signal
(c)
operon
reporter gene
mRNA
reporter protein
Signal

◀ **Fig. 2** Whole-cell-based biosensing system types. **a** In positive regulation of reporter gene expression, the regulatory protein is bound to the operon, preventing expression of the reporter gene. The analyte enters the cell and binds the regulatory protein, allowing for reporter gene expression. **b** In negative regulation of reporter gene expression, the analyte enters the cell and binds the regulatory protein. This analyte-regulatory protein complex can bind the operon and allow for expression of the reporter gene. **c** In constitutive expression, the reporter gene is constantly expressed

may prove difficult to express and purify, thus whole-cell biosensors provide an alternative route to investigating interactions with these proteins. Interestingly, bacterial whole-cell-based biosensors can provide physiologically relevant data and evaluate the bioavailability of the analyte of interest, inasmuch as the target chemical must be ingested by the cells in order to trigger a response. However, this requirement can also prove to be a limitation, as the bacteria rely primarily on diffusion, and thus may lack specific transport channels that may be necessary for the uptake of certain analytes.

2 QSMs in Physiological Samples via Bacterial Whole-Cell-Based Biosensing Systems

2.1 AHL Detection

Studies have shown that bacterial AHLs, such as 3-oxo-dodecanoyl-homserine lactone (3-oxo-C12-HSL), not only regulate bacterial virulence but can also stimulate cells important for inflammation and immune defense in the host [8]. It has been demonstrated that in burn wound mouse models infected with QS deficient bacterial strains, there was no production of mRNA for pro-inflammatory cytokines IL-6, TNFα, CGB, and TGFβ [9]. The opportunistic pathogen *Pseudomonas aeruginosa*, which employs QS to regulate virulence factor production and biofilm formation, plays an important role in chronic wounds, which are a large burden to the health system worldwide; *P. aeruginosa* is estimated to be present in up to 80 % of chronic wound cases. In addition, when *P. aeruginosa* is present, wounds are more severe and heal at a slower rate, which is thought to be caused by cell-to-cell communication with gram-positive bacteria, including *Staphylococcus aureus* [10–12].

The implications of bacteria in a variety of disorders have prompted researchers to investigate the role of QS in certain disease states by detecting QSMs in physiological samples. In an effort to elucidate the roles of QSMs in their natural environments, physico-chemical methods have been devised for their detection, including but not limited to high-performance liquid chromatography (HPLC), reversed phase HPLC mass spectrometry (RP-HPLC-MS), ultra-high-resolution mass spectrometry, and gas chromatography mass spectrometry (GC-MS) [13–15]. Additionally, work by Campagna et al. [16] and May et al. [17] has led to liquid chromatography–tandem mass spectrometry (LC-MS/MS) detection methods for

Table 3 An overview of bacterial whole-cell-based biosensors employed for detection of AHLs in physiological samples

Plasmid(s)	Sensor name	Bacterial strain	Detects	Promoter	Reporter
pSB401		*E. Coli*	Short-chain AHLs	*luxRI*	LuxCDABE
pSB1075		*E. Coli*	Long-chain AHLs	*lasRI*	LuxCDABE
pECP61.5		*P. aeruginosa*	Short-chain AHLs	*rhlA*	LacZ
pKDT17		*P. aeruginosa*	Long-chain AHLs	*lasB*	LacZ
pCF218, pMV26	A136	*A. tumefaciens*	Short- and long-chain AHLs	*traRG*	LacZ
pSB406		*E. Coli*	Short chain-AHLs	*rhlRI*	LuxCDABE
	CV026	*C. violaceum*	Short chain-AHLs	*luxI*-box-like	Violacein
	VIR07	*C. violaceum*	Long chain-AHLs	*cviR*	Violacein

AI-2 and AHLs in biologically relevant samples. Unfortunately, these methods require extensive sample extraction and/or preparation, large amounts of trained technician time, and expensive instrumentation, thus limiting their utility. Therefore we explore some of the findings from utilizing bacterial whole-cell-based biosensors for QSMs in physiological samples. The sensors employed for AHL detection are listed in Table 3. Please note, for the experiments and sensors described in the Table 3, short chain AHLs refer to AHLs with carbon chains of seven or less carbons, whereas long-chain AHLs refer to AHLs with carbon chains of eight or more carbons.

2.1.1 Cystic Fibrosis (CF)

Cystic fibrosis (CF) is an autosomal recessive genetic disorder that primarily affects the lungs, as well as the pancreas, liver, and intestine. Common symptoms include infertility, poor growth, sinus infections, and lung infections that lead to difficulty breathing. Although a large number of bacteria have been indicated to play a role in CF, the pathogenesis has yet to be fully elucidated [18]. Studies have shown that *P. aeruginosa* forms thick biofilms within the lungs of CF patients and uses QS extensively to modulate both biofilm formation and maturation [19, 20]. Of these bacteria, *P. aeruginosa* and *Burkholderia cepacia*, which are known to colonize the lungs of CF patients, both use AHL-dependent QS regulation [21].

In a study by Middleton et al. [22], sputum samples were collected from stable CF patients, extracted, and then analyzed by employing two *E. coli* whole-cell-based sensors bearing plasmids pSB401 and pSB1075, to detect short- and long-chain AHLs, respectively. For sputum samples colonized with *P. aeruginosa*, 71 % contained short-chain AHLs and 61 % contained long-chain AHLs ($n = 42$). Correspondingly, for sputum samples colonized with *B. cepacia*, 87 % contained short-chain AHLs and 50 % contained long-chain AHLs ($n = 8$). Another study

published the same year by Erickson et al. [23] further confirmed the presence of AHLs in sputum samples from CF patients. Whole-cell sensors based on *P. aeruginosa* carrying plasmids pECP61.5 and pKDT17 were utilized to detect short- and long-chain AHLs, respectively. Short-chain AHLs were found in 26 % of the patient sputum samples and long-chain AHLs were found in 78 % of the samples (n = 29). This finding is in contrast to the results of Middleton et al. [22], however there are several possible explanations: small numbers of different patients were assessed, different sample processing/extraction methods were used, and different sensing systems, both the bacterial strains and the plasmids employed, were used. Unfortunately, the small number of patients assayed, the variability of the patient disease status, and the lack of an established range of AHLs in sputum, do not allow for conclusions to be drawn on how and if AHL levels fluctuate in CF patient samples.

These works were followed by Chambers et al. [24], which focused on identifying a broad range of AHLs within mucopurulent respiratory secretions from CF patients undergoing lung transplantation. *Agrobacterium tumefaciens*-based whole-cell sensor A136 was employed, which contains dual plasmids pCF218 and pMV26, allowing for detection of both short- and long-chain AHLs. Mucopurulent material was extracted, separated by reversed-phase fast pressure liquid chromatography (FPLC), and then analyzed by sensor A136. By combining the whole-cell-based sensor with FPLC, AHLs were found in 69 % of patient samples, with seven unique AHLs being identified. These studies have helped to establish bacterial whole-cell-based biosensors as important tools for QSM detection, as well as have provided researchers a focal point for further discoveries to improve the lives and care of patients with CF.

2.1.2 Inflammatory Bowel Disease (IBD)

IBD is an autoimmune disorder that is characterized by a group of chronic inflammatory conditions of the gastrointestinal tract (GI), commonly causing severe abdominal pain, vomiting, diarrhea, rectal bleeding, and weight loss. IBD can be further classified based upon the symptoms and the extent of the inflammation within the GI tract, with Crohn's and Ulcerative Colitis being the two principal types. IBD is thought to be caused in genetically predisposed individuals by an overly aggressive immune response to commensal bacteria [25], thus research in this field has focused on the gut microbiome. The human gut is host to a broad range of bacteria, and although QS has been demonstrated for a limited number of intestinal bacteria, mainly pathogens, it is likely that a wide variety of enteric microorganisms, including commensal bacteria, communicate with each other and the host by means of QSMs.

Studies have shown that the bacteria of the gut play a role in IBD etiology, thus investigating QSMs in individuals may grant a greater understanding of the disease. To this end, work by Kumari et al. [15, 26] has led to the development and optimization of *E. coli* based whole-cell sensing systems, bearing plasmids pSB406

and pSB1075 [27], for quantitative detection of short- and long-chain AHLs (Fig. 3a, b), respectively, in human samples such as saliva and stool. By focusing on saliva and stool samples, this allows for a detection method that is completely non-invasive to patients. It is important to note that these methods do not require sample extraction or extensive sample preparation; simple centrifugation or dilution in water is all that is necessary to prepare samples. Additionally, these optimized systems allow for nanomolar limits of detection in physiological matrices, which makes these systems relevant, due to nanomolar levels of QSMs being necessary to initiate cellular communication [7]. Saliva from IBD and healthy individuals were assayed, revealing greatly varying levels of AHLs across individuals that were consistent for each individual for a period of time. Furthermore, stool samples from newborns admitted to a Neonatal Intensive Care Unit (NICU) also contained varying levels of AHLs. These results revealed for the first time that QSMs can be detected in human saliva and stool. These findings are of added interest, because it is well known that QSMs such as AHLs are subject to degradation by environmental factors, such as pH and temperature, and may be degraded by enzymes

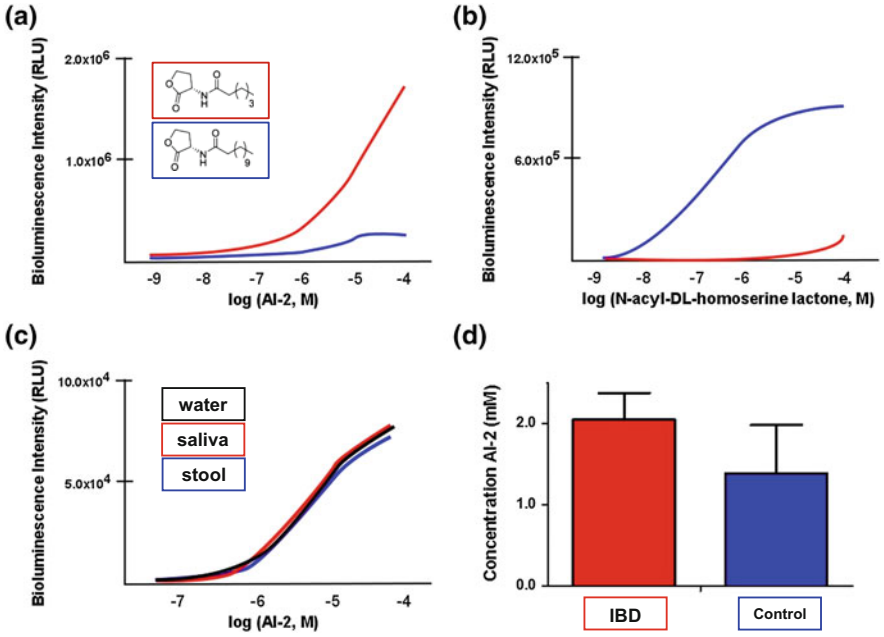

Fig. 3 Dose response curves generated for *N*-hexanoyl-DL-homoserine lactone (*red*) and *N*-dodecanoyl-DL-homoserine lactone (*blue*) on *E. coli* whole-cell-based biosensor harboring plasmid **a** pSB406 or **b** pSB1075, indicating the selectivity of each sensor for short or long chain AHLs (adapted from Kumari et al. [26]). **c** Dose response curves of AI-2 generated in water (*black*), 1:100 (v/v) diluted saliva (*red*), and 1:750 (w/v) stool (*blue*) with the *V. harveyi* BB170 sensor, indicating optimized sample dilutions for use with the sensor. **d** Saliva from IBD patients (*red*) and healthy controls (*blue*) were then diluted and assayed with the sensor, $n = 3$ (adapted from Raut et al. [30])

present in the gut [28, 29]. If a correlation can be established between the health of individuals and the levels of QSMs found in their samples, QSMs may develop into a biomarker for IBD, as well as other bacterial disorders. Current studies in the Daunert laboratory are in progress in which physiological samples from IBD patients, as well as matched controls, are being analyzed for their QSM levels, in order to investigate such correlation with disease status.

2.1.3 Hospital-Acquired Urinary Tract Infections (UTI)

Nosocomial infections (NI), commonly known as hospital-acquired infections, include multiple types of fungal and bacterial infections that are aggravated by the weakened resistance of patients. With *P. aeruginosa* known as a common link among many types of NI, multiple groups have worked to characterize the AHL profile of this microorganism during infection [31–33]. Gupta et al. examined the role of QSMs for their ability to induce pro- and anti-inflammatory renal cytokines and inflammation in an experimental urinary tract infection (UTI) model [34]. Their results suggested that deficiency for production of QSMs in mutant strains of *P. aeruginosa* resulted in non-stimulation of a pro-inflammatory response. Kumar et al. [31] employed sensors pKDT17, A136, CV026, and pECP61.5 to detect a broad range of AHLs: long-chain, both long- and short-chain, short-chain, and short-chain, respectively. Analysis of *P. aeruginosa* isolates from catheter-associated UTI found AHL molecules of varying types in all samples, indicating that the virulence factors activated may vary between UTIs. These findings were verified in work by Lakshmana Gowda et al. [33], who applied sensors pKDT17 and A136, not only to UTI isolates, but to other nosocomial infection *P. aeruginosa* isolates as well. However, work by Senturk et al. [32], which utilized sensors CV026 and VIR07 for detection of short- and long-chain AHLs, respectively, revealed another side to *P. aeruginosa* infection; although the diversity of AHLs seen previously was detected in most UTI isolates, 7 % contained no detectable levels of AHLs. Sequence analysis revealed mutations within genes crucial to the AHL pathway; *lasI*, *lasR*, *rhlR*, and *rhlI*. The authors hypothesized that these findings signify that *P. aeruginosa* possess additional virulence pathways that are independent of AHLs. Continued analysis of QSM levels in UTI isolates may help to elucidate the mechanism of *P. aeruginosa* infection, allowing for greater patient care and treatment.

2.1.4 Paper Strip Detection of Quorum-Sensing Molecules

The previously mentioned research on AHL detection has been focused on assays performed in the laboratory, however, when developing sensors that may have relevance for diagnostics or environmental detection, it is important to utilize a type of sensor and detection method which can be portable, that is, applied in a bedside or field kit. Whole-cell biosensors are ideal candidates due to their ability to withstand a range of environmental conditions, requirement of minimal or no

sample pretreatment, easy and rapid detection, and cost-effectiveness, to name a few [35, 36]. Due to the prevalence of QS in the pathogenesis of bacteria-related disorders, developing a fast, reliable, visual detection method for bacterial signaling molecules is of great interest. Work has been done to sense AHLs visually without instrumentation; however, these methods have shortcomings such as being time-consuming and not allowing detection in a dose-dependent manner [37]. To overcome these shortcomings, Struss et al. [38] designed a whole-cell biosensor that was amenable to paper strip incorporation for the semi-quantitative detection of bacterial quorum-signaling molecules. This whole-cell-based biosensor employed *E. coli* cells harbouring plasmid pSD908, which detects long-chain AHLs and contains the reporter gene *lacZ*, encoding β-galactosidase. β-galactosidase activity can be determined using a variety of substrates and, depending on the chosen substrate, detection of the analytical signal can be fluorescent, chemiluminescent, electrochemical, or colorimetric [26]. The *E. coli* cells harboring plasmid pSD908 were immobilized on the filter-paper-based strips by suspending the cells in a drying protectant solution and applying the suspension directly to filter-paper strips to allow them to dry via a liquid-drying method. This approach was used to help in the long-term preservation of cells. After the cells were dried on the filter-paper strips, they could then be immersed in liquid samples for detection of AHLs. After the sample immersion step, the strips were incubated in an 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) solution to develop a blue color. The intensity of the blue color increased with an increasing concentration of AHL, thus allowing semi-quantitative naked-eye evaluation (Fig. 4a). The paper strip sensor was employed for detection of AHLs in saliva and AHL levels were found in both healthy and diseased individuals (Fig. 4b), mirroring previous findings and proving the paper strip sensor's ability to detect AHLs in physiological samples. It is

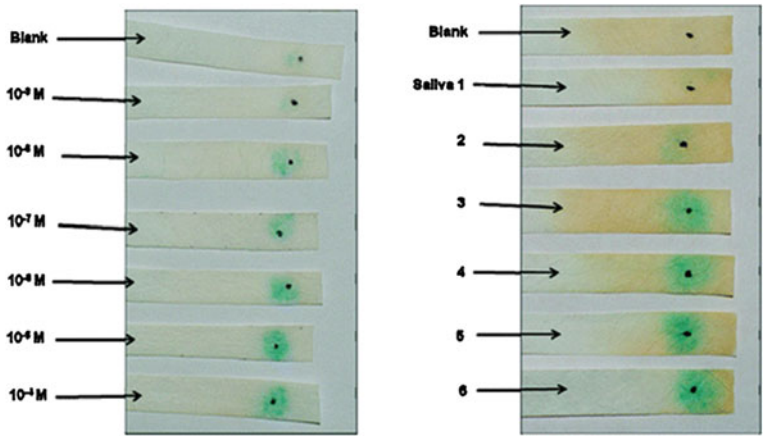

Fig. 4 **a** Dose-response data in buffer using filter-paper-based strips. The color intensity increases with increasing concentration of AHL. **b** Detection of AHLs in healthy patients (*1–5*) and a patient with Crohn's Disease (*6*). Reproduced with copyright permission from Struss et al. [38]

important to note that the assay was also performed at room temperature (RT), which demonstrates the applicability of the system for point-of-care diagnostics and field applications. Additionally, the paper strip sensors were found to be stable for at least three months at 4 °C without loss of analytical characteristics. Thus continued work with this and other bacterial whole-cell-based biosensors may allow for commercially available point-of-care and field tests for QSMs.

2.2 AI-2 Detection

In recent years, a group of molecules referred to as autoinducer-2 (AI-2) have been found in both Gram-positive and Gram-negative bacteria [39]. This finding is of great importance, as this indicates AI-2 molecules are used for interspecies communication. The production of these molecules from their common precursor, 4,5-dihydroxy-2,3-pentanedione (DPD), is catalyzed by the LuxS enzyme. In the organism *V. harveyi*, the AI-2 diffuses out of and into the cell and binds to a periplasmic binding protein, LuxP. This binding event starts a cascade of phosphorylation/dephosphorylation events and leads to the expression of luciferase for the production of light [40].

2.2.1 Inflammatory Bowel Disease (IBD)

As mentioned previously, AHLs are currently being investigated as a potential biomarker for IBD. As part of this research, Kumari et al. [26] and Raut et al. [30] hypothesized that monitoring the QSMs in physiological samples over time can give an idea of the amount of bacteria present, inflammation, and the progress of the disease. Specifically, as part of determining QSM profiles in patients, the interspecies communication molecule AI-2, which encompasses both Gram-negative and Gram-positive bacteria, could prove an ideal non-invasive biomarker for gastrointestinal disease.

Vibrio harveyi strain BB170 has been genetically modified to respond only to AI-2. Previously, this strain had only been used as a bioassay to screen cell cultures and identify bacteria able to produce AI-2. Raut et al. [30] developed and optimized a biosensing system based on *V. harveyi* BB170 and applied it for the quantitative detection of AI-2 in saliva, stool, and intestinal samples from IBD patients (Fig. 3c, d). As with their previous AHL research, a focus was placed on developing a non-invasive assay that did not require extensive sample preparation or extraction. With a similar process of simple centrifugation and dilution of samples in water, stool and saliva samples could be assayed for AI-2. Stool samples from IBD patients were then analyzed and found to exhibit considerably varied levels of AI-2. The authors hypothesized that these variations may reflect perturbations in the microflora of the inflamed intestines [41, 42]. AI-2 was also detectable in saliva samples from IBD patients via the BB170 biosensor in nanomolar concentrations, which correlates well

with the levels of AI-2 previously detected in human saliva via LC-MS/MS (244-965 nM) [16]. Thus continued work with the universal signaling molecule AI-2 may help us better understand and diagnose IBD.

3 Quorum-Sensing Agonists and Antagonists

As our knowledge of QSMs and their potential roles as biomarkers of disease states has expanded, interest has focused on molecules that interfere with the quorum-sensing network, either by acting as agonists or antagonists [43]. By blocking or mimicking the native quorum-sensing molecule's signal, more insight can be gained in the quorum-sensing molecular mechanism and it may be possible to interfere effectively with QS and modify the behavior of bacteria. These studies typically demonstrated that compounds analogous to the cognate quorum-sensing molecule (i.e. having a lactone ring but varying tail lengths, oxidation states, or saturation levels) induced weak to moderate gene expression [44]. Whole-cell biosensors have proven to be valuable tools to elucidate effects of structurally similar and dissimilar molecules on QS.

3.1 Antibiotics

Antibiotics are employed all over the world for the treatment and prevention of bacterial infection, however, their mechanisms of action are not fully elucidated. To this end, researchers have investigated the effects of antibiotics on QS pathways. Previous reports demonstrate that the antibiotic azithromycin was able to interfere with the regulation of the quorum-sensing network of *P. aeruginosa,* inhibiting virulence factors and biofilm formation [45]. These studies, however, only investigated the behavior changes of the native cells, such as motility and biofilm formation, but did little to investigate the mechanism of action of the antibiotic on the quorum-sensing system. To better understand how antibiotics are able to interact with the quorum-sensing network and screen for antibiotics that are able to interfere with QS, Struss et al. employed *E. coli* whole-cell biosensors harboring plasmids pSB406 and pSB1075 for the detection of short- and long-chain AHLs, respectively [26, 46]. The authors investigated three antibiotics that were commonly used in the treatment of IBD: ciprofloxacin, metronidazole, and tinidazole. Azithromycin was used as a control, in addition to exogenous AHL addition, as it has previously been shown to act as a quorum-sensing agonist/antagonist [47–49].

As expected, azithromycin was able to induce a dose-dependent response in both the pSB406- and pSB1075-based whole-cell sensors. Furthermore, this response occurred in both the presence and absence of exogenous cognate AHL. The antibiotics tested were also able to activate the systems in both whole-cell sensors, both in the presence and absence of the cognate AHL; however, the similarly

structured tinidazole and metronidazole activated the pSB1075 sensor to a greater extent than the pSB406 system. It is important to note that when the authors performed the studies with the whole-cell biosensor, they normalized all of the results to cell viability. This was to ensure that the changes observed were due to the interaction of the compound with the regulatory protein and not other interactions with the other components in the cells or due to a decrease in signal due to a decrease in the number of live cells. Another important result to note is that the concentrations of antibiotic that initiated an agonistic response were clinically relevant and included the peak plasma concentrations of the antibiotics.

The authors point out that their results are in contrast to previous studies reported for ciprofloxacin [50]. However, the previous studies were performed in *P. aeruginosa* PAO1 cells whereas the current study was performed using *E. coli* cells. This is an important demonstration of how whole-cell biosensors can allow studying the effects of molecules on a specific protein/promoter system, and in the native cells the effect could be due to other cell components. This was proven by a study showing that azithromycin did not directly affect the expression of QS-related genes, but decreased AHL production by lowering expression of genes upstream to the synthase ones [45].

Whole-cell biosensors are powerful tools, specifically for quorum-sensing regulatory proteins that are difficult to purify and elucidate the structure. The ligand binding domain (LBD) of LasR was isolated and a crystal structure was solved [51]. A study was performed to screen antibiotics for inhibitory QS activity. Specifically, azithromycin and ciprofloxacin were docked against the LBD of LasR. It was shown that these antibiotics do not bind to the AHL binding site of LasR but rather to a different site [50]. By using a whole-cell biosensor, it was shown that although these molecules may not be binding to the LBD, they still bind in such a way that they can cause a conformational change and allow for LasR binding to its promoter region.

4 Conclusions and Future Perspectives

QS is communication between bacteria that allows for the regulation of genes which control certain behaviors such as formation of biofilms, expression of virulence factors, allows for mobility, and bioluminescence, to name a few. This intricate network of cellular communication is dictated by signaling molecules and their associated regulatory proteins. Although there have been continued efforts to develop QSM sensors using regulatory proteins [52], due to the difficulty to express and purify these regulatory proteins, whole-cell biosensors are important to assist in the understanding of the mechanism of QS. Many QS bacteria are implicated in diseases, therefore the studies we have discussed in this chapter have been performed to understand the QS and how it is involved in the disease state. Diagnosis and treatment of a disease are paramount, however, there are instances that could potentially be prevented altogether with proper methods of detection: annually,

approximately 48 million people in the United States experience a foodborne illness, resulting in approximately 3000 deaths and an annual cost of illness estimated between US$51–77 billion [53]. Many of the agents involved in causing food borne illness are bacteria [54], with multiple studies having linked QSMs to food spoilage and activation of virulence factors [55]. By focusing on development and optimization of bacterial whole-cell-based biosensing systems for QSMs in foods, it may prove possible to prevent many cases of illness, as well as provide better diagnosis and treatment of severe cases.

Interspecies communication is a growing field of investigation as it plays an important role in large microbial communities. Work has been completed to detect AHLs in ecosystems where a range of bacteria would be present, such as in soil and the rhizosphere [56, 57]. As previously described, certain autoinducer molecules, such as AI-2, are used for interspecies communication. However, continued investigation of QSMs and QS networks is necessary as some organisms such as *Salmonella, Shigella* and *E. coli* cannot synthesize their own AHLs, yet these bacteria are able to communicate with other bacteria. It is hypothesized that this communication is facilitated via a receptor-like protein (SdiA) with amino acid sequences that are homologous to LuxR-type activators [58, 59]. The strain of *E. coli, EHEC*, which results in acute hemorrhagic diarrhea and can cause hemolytic uremic syndrome, appears to produce a thus far unidentified autoinducer termed AI-3. Clarke et al. identified the *E. coli* protein QseC that directly binds both AI-3 and epinephrine/norepinephrine [60]. This was one of the first studies demonstrating that mammalian signaling hormones can be detected by bacteria. AI-3 is thought to activate genes that are involved in intestinal colonization. Other organisms that produce AI-3 are *Shigella, Salmonella, Klebsiella, Enterobacter* and *Citrobacter*. Little is known about how these molecules interact with various quorum-sensing systems, thus whole-cell biosensors could lend insight about which regulatory proteins are important for this interspecies communication and the mechanism of action of these molecules.

Furthermore, QS has been implicated in interkingdom communication. In a novel 3-D co-culture model of epithelial cells and immune cells (monocytes/macrophages) it could be shown that the epithelial cells degrade the *P. aeruginosa* QSM 3-oxo-C12-HSL. This protective effect by the epithelial cells is thought to be caused through enzymatic degradation of these QSMs by the epithelial cells [61]. This study demonstrated that QSMs are sensed not only by bacteria, but also by eukaryocytes. Additionally, QSMs have been shown to cause an inflammatory response [8], which make them an attractive target for therapeutic interventions. Thus by continuing to employ bacterial whole-cell-based biosensors, research may be able to elucidate further how our bodies interact with our biome and affect disease.

Research employing bacterial whole-cell biosensors has proved great insight into the quorum-sensing mechanism. With these tools, it is possible to evaluate molecules and determine which chemical characteristics are important to agonize or antagonize quorum-sensing systems. This information will strongly impact the future of treatment for many of the diseases where QS is implicated, as well as broaden our knowledge of bacterial communication.

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