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Detection and Control of Indoor Airborne Pathogenic Bacteria by Biosensors Based on Quorum Sensing Chemical Language: Bio-Tools, Connectivity Apps and Intelligent Buildings

Claudia Ibacache-Quiroga, Natalia Romo, Rodrigo Díaz-Viciedo and M. Alejandro Dinamarca

Abstract

Nowadays, lifestyles and climate change lead people to spend long periods in indoors spaces, where reduced ventilation and artificial light favor the concentration and spread of airborne pathogenic microorganisms. Current procedures for microbiological air evaluation are based on air sampling coupled to traditional microbiological culture-dependent methods such as biochemical tests and molecular rDNA 16S sequencing. These techniques generate an important delay in the application of prevention and control measures. This chapter presents whole cell-based biosensors that are able to detect quorum sensing signaling molecules produced by airborne pathogenic bacteria as a tool for indoor air monitoring. Furthermore, a general biosensor model is proposed. In this model, in vivo biosensors technology can be connected to online applications (Apps), being part of intelligent buildings, in order to reduce airborne pathogenic bacteria concentration and dissemination.

Keywords: air microbiology, quorum sensing, biosensors, airborne pathogens, hyperconnectivity, pathogen control, intelligent buildings

1. Introduction

Legionnaire’s disease outbreak (1976) is a masterpiece that allows us to understand how the interaction between environment, pathogen and host can be influenced by lifestyle and
technology [1]. Nowadays, because human population continues to grow and people spend their time in confined and shared spaces, concentration and spread of microorganisms must be controlled to avoid infectious outbreaks produced by airborne pathogens.

In indoor spaces, airborne pathogens can be part of aerosols that are produced and disseminated by heating, ventilation, air conditioning or humidifier systems (HVAC) [2]. These systems can be found in several buildings, including shopping centers, hospitals, hotels, cinemas, supermarkets, educational centers, restaurants, houses, airports, cars, trains and buses. Based on the above building design, HVAC equipment and population density are factors that must be considered to avoid the spread of airborne pathogenic microorganisms. In addition, appropriate air microbial quality controls are necessary to reduce biological risks.

Current procedures for microbiological air quality evaluation (ISO 14698-1:2003) are based on passive or active air sampling methods [3]. Passive methods involve the exposition of a petri dish (containing a selected solid culture media) to the environment during an established period, while active methods consist of automatic air samplers with a culture medium that is exposed to a forced airflow. In both methods, samples are incubated in favorable conditions for microorganism (bacteria, yeasts or molds), during 24–72 h. These methods are suitable for the risk assessment through microbial quantification in air [colony forming units (CFU) count]; however, they are not adequate for pathogen identification, for which biochemical characterization, immunoassays and 16S rDNA amplification and sequencing are more accurate and adequate. Nevertheless, these time-consuming procedures generate a delay in the surveillance of microbial air quality. For this reason, it is necessary to consider other methods that are able to detect and identify pathogenic microorganisms in a more efficient and rapid manner. In this context, biosensors able to detect specific molecules produced by pathogenic microorganisms are a more precise and faster method for the detection of airborne pathogens.

In this chapter, we describe different biosensors (based on whole cell sensing-reporter systems) that are able to detect bacterial signaling molecules produced in a concentration-dependent manner by the quorum sensing (QS) cell-to-cell communication system. These signaling molecules called autoinducers (AI) are present inside bacterial cells as well as in the environment and can be specific according to producer strain. Since QS is present in different pathogenic bacteria like Acinetobacter baumannii, Klebsiella pneumonia, Legionella pneumophila, Pseudomonas aeruginosa and Streptococcus pyogenes, it is proposed that biosensors can be applied to develop new technologies for the detection of airborne pathogenic bacteria in indoor spaces. Furthermore, a general model for biosensor technology focused on the development of intelligent buildings is presented. The aim of this model is to reduce airborne pathogenic bacteria concentration and dissemination, in association with online applications (Apps).

2. Airborne pathogens and quorum sensing

2.1. Airborne pathogens and indoor spaces

In confined and shared spaces, the host-environment-pathogen equilibrium can be altered due to inadequate building design that leads to a reduced air renewal, limitation of natural
light and favors overcrowding, increasing microbial concentration and dissemination of airborne pathogenic bacteria. Figure 1 shows four different models of pathogen-environment-host interaction. When environment-host-pathogen interplay is at equilibrium, pathogenic microorganisms exist at low concentration in the environment due to physical-chemical or biological factors such as temperature, ultraviolet light, pH and water activity ($A_w$) (a). In certain conditions, in which biological risks should be reduced at minimum or eliminated, pathogens should get excluded from the host’s environment (b). This includes research facilities with biosafety level 3 or 4, and pharmaceutical facilities for production of vaccines, medical devices or parenteral nutrition. On the other hand, in confined or overcrowded spaces, a major biological risk is expected due to impact of the environment on pathogen-host interaction (c). In this condition, different strategies to reduce microbial concentrations and disseminations should be considered. These strategies include ventilation, heating, air conditioning and humidifiers systems, as well as high efficiency particulate air (HEPA) and ultra-low particulate air (ULPA) filters, UV lamps and sanitizers (aerosol). On the other hand, when all measures for air quality control fail, the loss of host-environment-pathogen equilibrium generates an infectious outbreak (d).

**Figure 1.** Host (H)-environment (E)-pathogen (P) interplay in different conditions. The schemes show four different interaction conditions between the host and the pathogen. In an ideal condition (a), pathogens have a low interaction with the environment and the host, even though it is circulating in the population and the environment. In (b), there is a restrictive condition in which for biosafety reasons, the pathogen must be excluded from the environment and the host. In (c) and (d), a model is shown for host-environment-pathogen interaction in indoor at low and high biological risk, respectively.
2.2. Quorum sensing and chemical signals

Quorum sensing is a cell-to-cell communication system that allows bacteria to act in a coordinate manner. This mechanism is based on the synthesis, release and detection of signal molecules, called autoinducers (AI), whose increase is in a cell-density dependent mode. When AI reaches a threshold concentration due to an increase in bacterial population, the autoinducer activates a transcriptional regulator that controls gene expression of genetic elements under QS regulation. The first report of QS was in 1979, when Nelson and Hasting described this communication system as a regulatory mechanism of bioluminescence in *Vibrio harveyi* [4]. Nowadays, three parallel quorum sensing mechanisms have been identified in *V. harveyi* as regulators of gene expression [5].

In Gram-negative bacteria, QS consists typically of an autoinducer synthase and transcriptional regulator protein that binds to the AI and regulates gene expression of target genes. The chemical structure of the AI can vary between microorganisms; nevertheless, the main AIs in Gram-negative bacteria are N-acyl homoserine lactones (AHL). Other autoinducers identified in Gram-negative bacteria include: autoinducer-2 (AI-2); cholera autoinducer CAI-1, diffusible signal factor (DSF), *Legionella* autoinducer (LAI-1), among others (for review, see Ref. [6]). In Gram-positive bacteria, two types of QS systems have been identified: a one-component system and a two-component QS system. In both systems, the autoinducers correspond to oligopeptides called autoinducer peptide (AIP) that are synthetized and secreted to the environment, where they suffer structural modifications. In the one-component QS system, extracellular AIPs are transported back into the cell through permeases and are recognized by a specific receptor in the cytoplasm that acts as a transcriptional regulator. The two-component system consists of a membrane-bound protein kinase that recognized AIP and activates the transcriptional regulator in the cytoplasm through its phosphorylation (for review, see Ref. [7]).

2.3. Quorum sensing in airborne bacterial pathogens and their autoinducers

Quorum sensing (QS) communication system is present in a diverse group of microorganisms from environmental to human pathogenic bacteria. In pathogenic bacteria, QS regulates the expression of virulence factors such as biofilm formation, enzyme production and secretion and antibiotic resistance [8, 9]. Regarding airborne pathogens, QS communication system is present in several airborne bacteria (*Table 1*), playing a role in virulence and pathogenesis. *Pseudomonas aeruginosa* is a Gram-negative opportunistic bacterium that causes healthcare-associated infections, including respiratory infections in immunodeficient patients. These infections are of major concern in patients with cystic fibrosis and severe burn injuries [10, 11]. The Centre for Disease Control and Prevention (CDC) estimated that *P. aeruginosa* causes 51,000 healthcare-associated infections per year in the United States. Due to antibiotic resistance that reaches 13% in the USA, these infections can become chronic and are associated with high mortality rates. In this pathogen, several virulence factors are under QS control: biofilm formation, pyoverdine synthesis and hemolysin production, among others [12, 13]. The major autoinducer molecules identified in *P. aeruginosa* QS are N-butyryl-L-homoserine lactone and N-(3-oxododecanoyl)-L-homoserine lactone [14, 15].
Acinetobacter baumannii is a Gram-negative pathogen associated with hospital-acquired infections. The ability of this pathogen to develop antibiotic resistance is a public health issue worldwide. Its main QS signaling molecule has been identified as N-3-hydroxy-dodecanoyl-homoserine lactone, and in this pathogen, QS regulates biofilm formation and the expression of drug-resistance genes [16–18].

Klebsiella pneumoniae is also a Gram-negative bacterium that causes nosocomial infections. This pathogen presents type 2 QS system and uses AI-2 (furanosyl borate diester) as autoinducer, and this system is involved in biofilm formation [19].

Legionella pneumophila is a Gram-negative opportunistic pathogen that, through inhalation, can cause Legionnaires’ disease, which is a severe type of pneumonia. This pathogen uses LAI-1 (3-hydroxypentadecane-4-one) as autoinducers for Legionella quorum sensing (Lqs) system [20]. In L. pneumophila, QS system regulates biofilm formation, and LAI-1 has been described to be involved in inter-kingdom communication with eukaryotic cells [21].

Streptococcus pyogenes is a Gram-positive microorganism that causes pharyngitis and other respiratory tract infections. In this pathogen, QS has been related to protease production, among other phenotypical characteristics. Despite S. pyogenes is a Gram-positive bacterium, recent studies have identified that it uses AI-2 as a signaling molecule in QS [22].

### 3. Biosensors for detections of quorum sensing signals molecules

Due to quorum sensing (QS), communication system allows bacteria to act in a coordinate manner, to coordinate gene expression and to have a greater impact on their host, and this system has become a new target for the development of antimicrobial therapies as well as for bacterial diagnosis and therapeutic purposes [23, 24]. In this context, a diverse number of biosensors have been designed and developed to identify QS communication signals called autoinducers (AIs).

<table>
<thead>
<tr>
<th>Airborne pathogen</th>
<th>Pathology</th>
<th>Main autoinducer(s) type</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Opportunistic infections</td>
<td>AHL*</td>
<td>[14, 15]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-oxo-AHL*</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>Pneumonia, bronchitis</td>
<td>AI-2*</td>
<td>[19]</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>Opportunistic infections</td>
<td>3-hydroxy-AHL*</td>
<td>[18]</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>Pharyngitis, cellulitis</td>
<td>AI-2*</td>
<td>[22]</td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>Legionnaire’s disease</td>
<td>LAI-1*** (3-hydroxypentadecane-4-one)</td>
<td>[20]</td>
</tr>
</tbody>
</table>

*AHL: acyl homoserine lactone.  
**AI-2: autoinducer-2.  
***LAI-1: Legionella autoinducer.

Table 1. Selected airborne pathogens with quorum sensing communication system.
3.1. Diversity of quorum sensing biosensors: accuracy, precision and sensibility for autoinducers detection

Biosensors are analytical bio-physicochemical-electronic devices that are able to detect and quantify analytes from a sample (for review, see Ref. [25]). The physical-chemical-electronic component of a biosensor is a detector and transducer able to capture a specific signal generated by the biological component when it is associated with its cognate analyte. The biological component of a biosensor can be whole cells (genetically modified microorganisms containing a genic construct based on a sensing-reporter system); proteins (enzymes, antibodies and antigens) or nucleic acids. To enhance the interaction with the analyte and detector-transducer unit, the biosensor can be encapsulated or adsorbed on inert supports. This chapter focuses on whole cell genetically modified microorganisms designed to detect chemical analytes that are produced by specific bacteria, specifically to detect chemical signals called autoinducers (AIs) produced by the cell-to-cell QS communication system.

3.1.1. Accuracy, precision and sensibility of quorum sensing whole cell biosensors

Accuracy of QS biosensors for pathogen detection depends on the specificity of each molecular sensor (regulatory protein) in response to its autoinducer (AI). In this context, there exist QS whole cell detection systems for acylated homoserine lactones (AHL) and their 3-oxo-AHL and 3-hydroxy-AHL derivatives that are able to differentiate between the length of the acyl chain. For example, *Chromobacterium violaceum* CV026 and pSB536 can detect short-chain AHL, while pSB1075 detects long-chain AHL. On the other hand, other biosensors detect furanosyl borate diester (AI-2) using genetically modified *Vibrio harveyi* strains that do not produce this autoinducer and do not present receptors for other QS systems. From this point of view, QS biosensors can be considered an accurate method; however, it should be noted that it is an indirect detection method for pathogens.

Regarding QS biosensor precision and sensibility, whole cell biosensors can be classified according to their reporter system, which are activated by the transcriptional regulator associated to the AI. Table 2 shows different biosensors, their phenotypes and detection methods. From these detection systems, luminescence (fluorescence or chemiluminescence) is considered a precise and highly sensitive method [26]. Both, signal and detection methods (luminometer or spectrofluorimeter), allow to detect low concentrations of its AI, which is of special interest due to AI and can activate QS system at low concentrations. For example, threshold concentration of 3-oxo-N-acyl homoserine lactone for the activation of QS system in *P. aeruginosa* is 10 nM [27]; therefore, it is of extreme importance that biosensors can detect AI concentrations of this order of magnitude. In this context, QS biosensor can detect QS signaling molecules at concentrations ranging from pM to μM [28].

Table 2 shows different types of biosensors for the detection of quorum sensing signaling molecules and the reporter systems used in each case.

As previously described, AIs can diffuse outside the cell into culture medium (environment) and be sensed by other microorganisms. Figure 2 shows *C. violaceum* CV026 (A) exposed to AHL.
Table 2. Biosensor for quorum sensing signaling molecules.

<table>
<thead>
<tr>
<th>Biosensor</th>
<th>Host</th>
<th>Detected signaling molecule</th>
<th>Reporter system</th>
<th>Genotype</th>
<th>Phenotype</th>
<th>Detection method</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chromobacterium violaceum CV026</strong></td>
<td><strong>Escherichia coli</strong> JM109</td>
<td>C4-AHL, C6-AHL*, C6-3-oxo-AHL, C8-AHL, C8-3-oxo-AHL</td>
<td><strong>vioABCD</strong></td>
<td>C6-AHL</td>
<td>Violacon synthesis, Color</td>
<td>Colorimetric</td>
<td>[29]</td>
</tr>
<tr>
<td><strong>pSB401</strong></td>
<td><strong>Escherichia coli</strong> JM109</td>
<td>C6-AHL, C6-3-oxo-AHL, C8-AHL, C8-3-oxo-AHL</td>
<td><strong>luxCDABE</strong></td>
<td>C6-AHL</td>
<td>Luciferase synthesis, Luminiscence</td>
<td>Luminiscence</td>
<td>[30]</td>
</tr>
<tr>
<td><strong>pSB536</strong></td>
<td><strong>Escherichia coli</strong> JM109</td>
<td>C4-AHL*</td>
<td><strong>luxCDABE</strong></td>
<td>C4-AHL</td>
<td>Luciferase synthesis, Luminiscence</td>
<td>Luminiscence</td>
<td>[31]</td>
</tr>
<tr>
<td><strong>pSB1075</strong></td>
<td><strong>Escherichia coli</strong> JM109</td>
<td>C10-3-oxo-AHL, C12-3-oxo-AHL, C12-AHL</td>
<td><strong>luxCDABE</strong></td>
<td>C10-AHL</td>
<td>Luciferase synthesis, Luminiscence</td>
<td>Luminiscence</td>
<td>[30]</td>
</tr>
<tr>
<td><strong>pZLR4</strong></td>
<td><strong>Agrobacterium tumefaciens NT1</strong></td>
<td>C8-3-oxo-AHL*, C8-3-hydroxy-AHL, C6-AHL, C8-AHL, C10-AHL, C12-AHL, C14-AHL, C6-3-hydroxy-AHL, C8-3-hydroxy-AHL, C10-3-hydroxy-AHL, C12-AHL, C12-AHL</td>
<td><strong>lacZ</strong></td>
<td>C8-AHL</td>
<td>β-galactosidase activity, Color</td>
<td>Colorimetric</td>
<td>[32]</td>
</tr>
<tr>
<td><strong>pAS-C8</strong></td>
<td><strong>Broad host range</strong></td>
<td>C8-AHL, C10-AHL</td>
<td><strong>gfp</strong></td>
<td>C8-AHL</td>
<td>GFP synthesis, Fluorescence</td>
<td>Fluorescence</td>
<td>[33]</td>
</tr>
<tr>
<td><strong>Vibrio harveyi JMH597</strong></td>
<td></td>
<td>C10-AHL</td>
<td><strong>luxCDABE</strong></td>
<td>AI-2</td>
<td>Luciferase, Luminiscence</td>
<td>Luminiscence</td>
<td>[5]</td>
</tr>
</tbody>
</table>

*Major sensibility to this signaling molecule.
produced by \textit{C. violaceum} wild type (B), inducing violacein synthesis in strain CV026 as a positive reaction for the detection of AHL. On the other hand, \textit{A. tumefaciens} NT1 pZLR4 supplemented with X-gal shows no β-galactosidase activity (colorless) in the absence of AHL (A), while bacterial culture shows a chromogenic reaction when it is exposed to AHL due to this enzyme activity.

4. Choosing the appropriate biosensor phenotype for an indoor detection system

4.1. Quorum sensing microbial-based biosensors

Classically, quorum sensing (QS) has been studied to find new strategies to fight bacterial infections [34]; nevertheless, this system has also been proposed as a biomarker system [35]. Due to QS, autoinducers (AIs) are chemically diverse and are biomolecules produced under conditions by specific bacteria, and detection of AI allows an indirect identification of bacterial pathogens [36, 37]. Because AI concentration increases in a cell-density dependent manner, their detection and quantification also permit to determine the state of infection [38]. Several analytical methods have been used to identify these molecules, like ultra-performance liquid chromatography (UPLC), high-performance liquid chromatography (HPLC) and high-resolution mass spectrometry; nevertheless, these chemical analyses require high-tech equipment as well as sample preparation, extraction and purification [39]. Therefore, it has been proposed that QS microbial biosensors are a potent tool for environmental and healthcare monitoring [40]. Unlike biosensors for inorganic bacterial compounds like ATP [41], biosensors based on QS show higher specificity and consist of viable microbial cells.

4.2. Choosing the adequate quorum sensing biosensor

In order to detect airborne bacterial pathogens in indoor spaces in a more efficient manner, whole cell and cell-free biosensors are able to detect QS signaling molecules, which are of great
interest. These sensors are suitable for in vitro and in situ measurements [42]. As described earlier, reporter methods include luminescent, fluorescent and colorimetric signals (Table 2), which required widely available equipment for laboratory usage as well as for in situ measurements [43]. Additionally, QS biosensors can detect QS signaling molecules at low concentrations, ranging from pM to μM [28]. Figure 3 shows a biosensor model for autoinducer detection. This biosensor is composed of four essential genetic elements: promoter R1, gene encoding QS transcriptional regulator, promoter R2 and a reporter system. R1 is a promoter region that regulates gene expression of the transcriptional regulator of QS system. This promoter can be designed in order to respond to different stimuli and induce gene expression of the transcriptional regulator. This transcriptional regulator binds the autoinducer and regulates gene expression of the reporter system by binding promoter region R2, which is a QS promoter region.

The main issues regarding detection of airborne pathogens are related to low bacterial concentration in air samples and interference of other particulate materials in the analyses, requiring appropriate sampling methods and equipment. In this context, QS biosensor technology should contain three essential units: (i) air sampler, (ii) cassette containing active bacterial cells used as biosensor and (iii) a signal processing module that allows data analysis and report generation. There are two main strategies to obtain air samples: (1) to use air samples and (2) harvest particulate matter from air conditioning equipment [44]. Air samples can be

Figure 3. Biosensors for detection of autoinducers (AI) molecules from quorum sensing (QS). Promoter R1 regulates gene expression of the quorum sensing transcriptional regulation (TR) that binds autoinducer (AI) molecules. The TR-AI complex induces gene expression of the reporter system by binding promoter region R2, which is a canonical QS promoter region.
directly coupled to culture medium or inorganic supports containing the biosensor, which will be activated in the presence of QS signaling molecules [45].

The selection of the appropriated biosensor will depend on equipment availability. Colorimetric biosensors do not need a specialized instrument for qualitative analysis due to their visual signal. In case of a quantitative evaluation, a spectrophotometer equipped with specific filters is needed. On the other hand, luminescent and fluorescent biosensors require luminometer and a fluorimeter, respectively, for qualitative and quantitative measurements. Excitation and emission wavelength will depend on the fluorescent protein, which is used as a reporter.

5. A model for future developments: integrating biosensors to global connectivity era and intelligent building to reduce indoor microbiological risks

From a positive and holistic point of view, the vertiginous advances in connectivity, robotics, automation, electronics, computer science, synthetic biology and artificial intelligence allow us to understand that these disciplines will improve our living conditions. In this context, it is easy to imagine the positive impact of automated bioelectronic systems integrated into architecture design and newly build techniques on life quality and health. However, the most revolutionary aspect will be incorporation of intelligent automation devices in cars, houses, hospitals, classrooms or institutional buildings, and how these systems will intelligently generate favorable healthy conditions for the people, cities and their environments [46].

On the other hand, considering climate change and the increase in antibiotic resistance, complex solutions should be developed to avoid health problems associated with indoor spaces such as the sick building syndrome (SBS) [47, 48]. In this context, the integration of biosensors for the detection and surveillance of pathogenic microorganisms and quality control indoor spaces is an appropriate challenge [49–52].

Synthetic biology is an interdisciplinary tool based on biology, engineering and bioinformatics that appears appropriate to generate a bridge to connect bio-based solutions with indoor microbial air quality systems in intelligent buildings. For example, with this tool, it is possible to develop genetic circuits and new bioelectronic devices for the detection of pathogens [40, 53]. As previously discussed, biosensors (cell-based or cell-independent sensors) are a suitable tool for the detection of molecules related to environmental quality problems or health risks. In this sense, the development of new bioelectronic devices that consider a sampler unit, a biosensor unit and a receptor unit, remotely connected through online systems represents an advance that allows us to efficiently act against pathogens in indoor environments. In this context, it is important to highlight that the main advantage of smart buildings for human health is related to their ability to couple air quality sensors with automatic control systems.

According to the abovementioned factors, Figure 4 shows an integrative model of biosensors coupled to an air sampler, equipped with units that allow (i) capturing microorganisms and
their molecules, (ii) exposing them to biosensors, (iii) capturing the signals emitted by the bio-
sensor and (iv) analyzing them and sending a report through web applications to the users. 
Likewise, the proposed model integrates this technology into intelligent buildings or indoor 
spaces in general to remotely activate automated systems that reduce the microbial load or 
informs the health authority in the event of an infectious outbreak occurs.

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**Figure 4.** Sensors for detecting and monitoring pathogens in indoor spaces in the era of connectivity, intelligent buildings and automation. Figure shows the integration of a quorum sensing autoinducer biosensor to an intelligent air sampling system, connected to an intelligent control and surveillance system.
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