Comprehensive REVIEWS in Food Science and Food Safety

Bacterial Quorum Sensing and Food Industry

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Abstract: Food spoilage and biofilm formation by food-related bacteria are significant problems in the food industry. Even with the application of modern-day food preservative techniques, excessive amounts of food are lost due to microbial spoilage. A number of studies have indicated that quorum sensing plays a major role in food spoilage, biofilm formation, and food-related pathogenesis. Understanding bacterial quorum-sensing signaling systems can help in controlling the growth of undesirable food-related bacteria. This review focusses on the various signaling molecules produced by Gram-negative and Gram-positive bacteria and the mechanism of their quorum-sensing systems, types of signaling molecules that have been detected in different food systems using biosensors, the role of signaling molecules in biofilm formation, and significance of biofilms in the food industry. As quorum-sensing signaling molecules are implicated in food spoilage, based on these molecules potential, quorum-sensing inhibitors/antagonists can be developed to be used as novel food preservatives for maintaining food integrity and enhancing food safety.

Practical Application: Bacteria use signaling molecules for inter- and intracellular communication. This phenomenon of bacterial cell-to-cell communication is known as quorum sensing. Quorum-sensing signals are implicated in bacterial pathogenicity and food spoilage. Therefore, blocking the quorum-sensing signaling molecules in food-related bacteria may possibly prevent quorum-sensing-regulated phenotypes responsible for food spoilage. Quorum-sensing inhibitors/antagonists could be used as food preservatives to enhance the shelf life and also increase food safety.

Introduction

Food spoilage is a complex process. It is caused by the various biochemical changes naturally occurring in foods and due to microbial activities. Microbial spoilage is the most common cause of spoilage (Gram and others 2002). Excessive amount of foods are lost due to microbial spoilage. It is a major concern in the food industry as it causes significant economic losses and can have serious public health consequences (Kumar and Anand 1998). Microbial food spoilage manifests itself as visible growth and food textural changes (Gram and others 2002).The off-taste and off-odor due to release of metabolites by microbes results in rejection of the food. Microorganisms produce saccharolytic, proteolytic, pectinolytic, and lipolytic enzymes whose metabolic end products are associated with food spoilage (Braun and others 1999; Loureiro 2000; Ragaert and others 2007). Thus, microbial activity is considered to be of great importance for the manifestation of spoilage (Nychas and others 2006, 2007). In recent years, the detection of quorum-sensing signals in spoiled food products has added a new dimension to study the process of food spoilage. Quorum sensing or cell-to-cell communication is employed by a diverse group of bacteria including those commonly associated with food to communicate with each other by producing the signaling molecules, autoinducers. Through the mechanisms of quorum sensing, bacteria are able to express specific genes in response to population density. It is possible that the bacterial spoilage of some food products is influenced by quorum-sensing-regulated phenotypes. Further studies on the possible role of quorum-sensing compounds in food spoilage reactions can lead to the development of novel preservation techniques based on potential quorum-sensing inhibitors.

Quorum sensing has been extensively studied in bacterial pathogenicity (Smith and others 2004), and now it is implicated that quorum sensing could be involved in bacterial food spoilage. Several proteolytic, lipolytic, chitinolytic, and pectinolytic activities associated with the deterioration of foods are regulated by quorum sensing. Moreover, several types of signaling molecules have been detected in different spoiled food products. Hence, disrupting the quorum-sensing circuit can play a major role in controlling microbial gene expression related to human infection and food spoilage. The role of quorum-sensing signaling molecules involved in food spoilage needs to be understood to block the causative cell-to-cell communication and prevent microbial spoilage. Quorum-sensing inhibitors can be developed that target synthesis of the cell signaling molecules or block these signaling systems that can lead to prevention of food spoilage and biofilm formation by food-related bacteria.

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doi 10.1111/j.1541-4337.2011.00150.x

The aim of this review is to understand the mechanism of quorum sensing in food spoilage and by foodborne bacteria, assess the role of signaling molecules in food spoilage and pathogenicity of food-related bacteria, and to exploit potential quorum-sensing inhibitors as food preservatives to delay or prevent food spoilage.

Quorum Sensing

One of the most remarkable discoveries in microbiology in the past 30 y has been the fact that bacterial cells communicate with each other. In bacteria, cell-to-cell communication is a wide spread phenomenon controlling a broad range of activities. The modulation of gene expression in bacteria by quorum sensing results in phenotypic changes leading to their better adjustment to environmental conditions during growth (Turovskiy and others 2007). Cell-to-cell communication depends on the production of, secretion of, and response to small, diffusible signal molecules called autoinducers. The signal molecules are produced and secreted at a basal level during bacterial growth. Their concentration in the environmental medium or matrix increases as the bacterial population expands, and when it reaches a threshold level (quorum level), it induces phenotypic effects by regulating quorum-sensing-dependent target gene expression (Czajkowski and Jafra 2009). This phenomenon occurs without any external intervention and is also referred to as autoinduction (Nealson and others 1970). The cell-to-cell communication has been aptly termed quorum sensing by Fuqua and Winans (1994). The various processes modulated by quorum sensing are usually tuned for damage to bacteria and their survival under stressful conditions. Quorum sensing is involved mainly in the regulation of virulence, development of genetic competence, transfer of conjugative plasmids, sporulation, biofilm formation, antimicrobial peptide synthesis, and symbiosis (Smith and others 2004).

There are 2 groups of signal molecules involved in bacterial quorum sensing. One is the peptide derivatives typically used by Gram-positive bacteria, while the fatty acid derivatives are exploited by the Gram-negative bacteria. Quorum sensing is omnipresent in many known bacterial species. Many human and plant pathogenic Gram-negative bacteria, including the genera *Agrobacterium*, *Brucella*, *Bukholderia*, *Erwinia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia*, *Vibrio*, and *Yersinia* exploit the quorum-sensing mechanism for the regulation of virulence factors syntheses (Williams 2007). Bacteria from the genera *Bacillus*, *Enterococcus*, *Staphylococcus*, *Streptococcus*, and *Streptomyces* utilize this mechanism to develop genetic competence, produce antimicrobial peptides or exotoxins, and for biofilm formation (Podbielski and Kreikemeyer 2004). The *Rhizobium* genus uses quorum sensing for nitrogen fixation. In these symbiotic bacteria, the nodulation and symbiosome development required for nitrogen fixation is under complex quorum-sensing regulatory systems (Hoang and others 2004). Quorum sensing has also been observed in extremophiles such as the haloalkaliphilic archeon *Natronococcus occultus* and the *Halomonas* genus of bacteria (Paggi and others 2003; Llamas and others 2005), in the hyperthermophilic bacterium *Thermotoga maritima* (Johnson and others 2005), and in *Acidithiobacillus ferrooxidans* (Rivas and others 2007), an acidophilic bacterium.

Mechanisms of Quorum Sensing

Most bacteria utilize 2 general mechanisms for detecting and responding to quorum-sensing signals. These 2 quorum-sensing systems are used in modulating the target gene expression. One mechanism is represented by acylhomoserine lactone (AHL) dependent quorum-sensing systems. Here, the quorum-sensing signal is detected by a cytosolic transcription factor. In the other mechanism, the quorum-sensing signal, such as the autoinducing peptide (AIP) produced by *Staphylococcus aureus*, is detected by a membrane-associated 2-component response regulatory system (Dong and others 2005). However, the quorum-sensing circuit of *Vibrio harveyi* has features of both the Gram-negative and Gram-positive quorum-sensing systems. Similar to other Gram-negative bacteria, *V. harveyi* produces and responds to acylated homoserine lactone (HSL), but analogus to the Gram-positive bacteria the quorum-sensing signal transduction occurs by the membrane-bound histidine kinases (Waters and Bassler 2005).

In AHL-mediated quorum sensing, bacteria synthesize AHL molecules using S-adenosylmethionine (SAM) and acyl chains derived from the common fatty acid biosynthesis pathway. AHL synthase (I-protein) encoded by LuxI homologue synthesizes AHL molecules (More and others 1996; Schaefer and others 1996). During low bacterial population density, each cell produces a basal level of AHL signal. The short-chain AHL signal passively diffuses across bacterial membranes and accumulates in the environment (Pearson and others 1999; Dong and others 2005). But the long-chain AHL signals require active transportation mechanisms for their efflux (Chan and Chua 2005). One such example is *Pseudomonas aeruginosa* that uses multidrug efflux pump MexAB-OprM for the active transport of 3-oxo-C12 HSL signal (Pearson and others 1999). With an increase in bacterial population, the concentration of AHL signal reaches a threshold level, resulting in signal accumulation and recognition by the cognate receptors. The signal reception involves R protein. These R proteins belong to the Lux R family of transcriptional regulators and act as receptors for the AHLs synthesized by the LuxI proteins. The R-AHL complex, which is a dimer, binds to conserved palindromic sequences of the quorum-controlled promoters, including the promoter of the luxI-type gene, and boosts AHL production (autoinduction) and expression of other genes in the quorum-sensing regulon (Schuster and others 2004). Thus, the R-AHL complex is involved in autoinduction and control of quorum-sensing regulons. The AHL-degradation enzyme and the cognate regulatory transcription factor(s) are involved in signal decay. The AHL-degradation enzymes have been identified in several bacterial pathogens that produce AHL signals, such as *Agrobacterium tumefaciens* and *P. aeruginosa* (Huang and others 2003). The degradation of AHL signals switches off the quorum-sensing-dependent gene expression (Zhang and others 2004).

AHL-mediated quorum sensing is one of the best characterized cell-to-cell communication mechanisms (Table 1). It is used by many bacterial species among which are, the agriculturally important *A. tumefaciens* and *Erwinia carotovora* and the medically important *P. aeruginosa* and *Burkholderia* species (Dong and others 2000; Williams 2007).

The 2-component system mediated quorum sensing is present in both Gram-positive and Gram-negative bacteria (Waters and Bassler 2005). Here, the quorum-sensing signal, such as the AIP signal, is transported to the intercellular environment by an ABC transporter. The accumulated signals are detected by a 2-component sensor that transfers the sensory information to activate a cognate response regulator for modulating the expression of quorum-sensing regulon through regulatory RNAs and intracellular transcription factors (Novick 2003). There are no reports on signal decay in the AIP-type quorum-sensing process.

Table 1–Mechanism of AHL-type quorum-sensing systems.

Quorum sensing process	Outcome	References
At low population density, AHL synthase uses S-adenosylmethionine (SAM) and acyl chains to synthesize AHL.	Signal generation	Schaefer and others (1996)
Short-chain AHLs diffuse passively. Long-chain signals require active efflux.	Signal accumulation	Dong and others (2005)
At high population density, LuxR-type (R) transcription factor recognizes signal.	Signal reception	Schuster and others (2004)
R proteins and AHLs bind to target DNA leading to increased AHL signal production and activation of quorum-sensing regulon.	Autoinduction	Zhu and Winans (1999)

Quorum-Sensing Signals

Cell-to-cell signaling systems are broadly grouped into 4 main categories. Two of these systems, which use autoinducer-1 (AI-1) and autoinducer-3 (AI-3), are found in Gram-negative cells, while the Gram-positive bacteria use a 3rd type of signaling system, the autoinducing polypeptide (AIP) system. These are primarily involved in intraspecies communication (Smith and others 2004). The 4th system, using autoinducer-2 (AI-2), is found in Gram-positive as well as Gram-negative bacteria. Quorum sensing using AI-2 is used for interspecies communication (Schauder and others 2001). All of these systems begin with production and release of autoinducer into the environment by the bacteria. The detection of these chemically distinct autoinducers, and the resulting alteration in gene expression, is specific to each system.

Autoinducer-1

The LuxIR system was first discovered in *Vibrio fisheri* during the investigation of the phenomenon of bioluminesence (Nealson and others 1970). Now, the LuxI/LuxR system has become the model system upon that the other quorum-sensing systems have been based. It consists of LuxI, which synthesizes an N-AHL called AI-1, and LuxR, a transcription factor responsible for controlling gene expression in the presence of the autoinducer. LuxI and its homologues synthesize autoinducers by transferring a fatty acid chain from an acylated acyl carrier protein (ACP) to SAM, releasing AHL and methylthioadenosine (Schaefer and others 1996). AHLs produced by LuxI homologues possess different fatty acid moieties due to recognition of specific ACPs by the synthases and result in intraspecies and intrageneric signals. Once synthesized, AI-1 diffuses across the bacterial membrane and is released into the surrounding environment. With an increase in population, the concentration of AI-1 in the environment rises. At high population density, the local concentration of AI-1 is high enough to diffuse back into the cell, where it binds to LuxR. When bound to AI-1, LuxR activates the transcription of the *luxCDABEGH* operon by binding Lux boxes located within the promoter (Devine and others 1989). The product of this operon, luciferase, catalyzes a chemical reaction that results in luminescence.

Homologous LuxI/LuxR systems have been identified in many Gram-negative bacteria, each capable of producing specific AHLs. In the opportunistic pathogens, such as *P. aeruginosa* and *Serratia marcescens*, these signaling mechanisms control the expression of the virulence factors. *Pseudomonas aeruginosa* contains 2 systems homologous to LuxI/LuxR. LasI/LasR has been shown to control biofilm formation and the production of extracellular enzymes, as well as transcription of another quorum-sensing system, RhlI/RhlR, adding an additional level of control through AHL signaling (de Kievit and Iglewski 2000). Earlier it was assumed that bacteria use quorum-sensing systems to monitor their population density, but studies on *Escherichia coli* (*E. coli*) and *Salmonella* Typhimurium show that this is not always the case. EC and *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), for example, do not have a LuxI homologue and therefore do not produce any AI-1, but they do encode a LuxR homologue named SdiA that when overexpressed has been shown to have a negative effect on genes involved in cell attachment in enterohemorrhagic EC (EHEC) (Kanamaru and others 2000), but positively regulates several genes located on the *S.* Typhimurium virulence plasmid including *rck*, a protein implicated in evasion of the host immune response (Ahmer and others 1998). Although the exact role of SdiA in pathogenesis is unclear, this protein allows EHEC and *S.* Typhimurium to alter gene expression in response to the presence of AI-1 produced by other bacteria (Michael and others 2001).

Autoinducer-2

Many Gram-negative and Gram-positive bacteria possess quorum-sensing systems that detect an extracellular signal named AI-2. This signal is synthesized from a by-product of SAM metabolism. For example, in *V. harveyi* synthesis of AI-2 is by the protein LuxS, a synthase encoded by luxS genes. LuxS synthesizes AI-2 from SAM through a series of steps involving conversion of ribosehomocysteine into homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD), a compound that cyclizes into several furanones in the presence of water (Schauder and others 2001). The structure of the 2 AI-2 signals has been determined by cocrystallization with 2 different AI-2-binding proteins: a furanosyl borate diester (BAI-2) used by *V. harveyi* to control luminescence and a furanone ([2R,4SL]-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran [R-THMF]) used by *S.* Typhimurium (Miller and others 2004).

AI-2 released by the bacterium accumulates in the cell's environment and its detection can be by 2 separate mechanisms. In the case of *V. harveyi*, it detects the BAI-2 form of AI-2. This mechanism detects the presence of AI-2 in the periplasm by first binding the signal with an autoinducer-specific binding protein, LuxP. This AI-2/LuxP complex then interacts with a sensor kinase, LuxQ, initiating a phosphotransfer cascade that results in luciferase production and luminescence. So far, the LuxP/LuxQ cascade has been identified only in *Vibrio* spp. AI-2 is handled by a different mechanism in *E. coli* and *S.* Typhimurium. Unlike the LuxP/LuxQ system, the Lsr (LuxS regulated) system induces a cellular response by transporting AI-2 into the cytoplasm of the cell. This process starts with recognition of the signal by a periplasmic protein, LsrB, which binds the R-THMF form of AI-2. Once bound, the Lsr ABC transporter, comprised of LsrA and LsrC, imports AI-2 into the cell where LsrK phosphorylates it. The phosphorylated form of AI-2 interacts with the transcriptional repressor LsrR to relieve repression of the *lsr* operon that may upregulate additional operons in the presence of phosphorylated AI-2 (Taga and others 2001).

LuxS/AI-2 systems have been discovered in a wide range of Gram-positive and Gram-negative bacteria leading to the suggestion that the AI-2 system is used for cross-species signaling by organisms living in mixed-species communities such as biofilms (Xavier and Bassler 2003).

Autoinducer-3

AI-3 employed by the QseC system was first described as a compound found in spent media that activated the expression of

genes involved in attachment of EHEC to, and subsequent actin rearrangement in, eukaryotic cells (Sperandio and others 2003). The structure and synthesis of this signal is still not clear. Early data suggested that LuxS was involved in AI-3 synthesis, as AI-3 production is impaired in *luxS* mutants. Further studies, however, proved that the lack of AI-3 production in these mutants was due to the use of oxaloacetate, instead of SAM, as a precursor for methionine. The addition of L-aspartate to the growth medium relieved the demand for oxaloacetate, and restored AI-3 production and had no effect on AI-2 production (Walters and others 2006). This study also found that a number of commensal bacteria such as nonpathogenic EC and *Enterobacter cloacae*, as well as pathogenic *Shigella*, *Salmonella*, and *Klebsiella* species, all produce AI-3. This suggests that AI-3 might represent another cross-species signal. However, this new signal has not yet been detected in Gram-positive bacteria.

Detection of AI-3 is accomplished through a 2-component system comprised of the sensor kinase QseC and response regulator QseB. In the presence of periplasmic AI-3, QseC undergoes autophosphorylation and transfers the phosphate to QseB, which activates genes responsible for flagella biosynthesis and motility by upregulating the master flagellar regulator gene *flhDC* (Clarke and others 2006). The presence of AI-3 is also linked to the formation of attaching and effacing lesions by EHEC, a feat accomplished through upregulation of 5 separate loci of enterocyte effacement (LEE) operons located within the EHEC chromosome (Sperandio and others 2003). The complete cascade responsible for regulation of these genes remains unclear, but likely involves QseA, a LysR-family regulator that is influenced by cell-to-cell signaling and directly upregulates LEE genes (Sperandio and others 2002).

Autoinducing Peptides

AIP for cell-to-cell signaling is found exclusively in Gram-positive bacteria and are based on the prototypic Agr system first identified in *S. aureus*. The Gram-positive bacteria use a polypeptide signal instead of a smaller molecule. The polypeptides produced by the Gram-positive bacteria act as an autoinducer for the organism that produces it but inhibits other organisms. This signal is referred to as an AIP and is encoded by the *agrD* gene. After translation, the AgrD propeptide is targeted to the membrane by an N-terminal signal sequence. Once at the membrane, AgrB, a membrane-bound endopeptidase, cleaves the C-terminus of the propeptide. The N-terminus of the propeptide, including the signal sequence, is removed by the signal peptidase SpsB. Finally, the C-terminus of this processed polypeptide is covalently linked to a conserved, centrally located cysteine to form a thiolactone ring with a free N-terminal tail. In many cases, both these structures are required for proper functioning of the AIP. After its release into the environment, the AIP is recognized by a signal receptor, AgrC. This protein contains an N-terminal transmembrane domain responsible for recognizing specific AIPs and a C-terminal histidine kinase domain that, in the presence of the correct AIP, phosphorylates a response regulator called AgrA. Phosphorylated AgrA activates transcription of select genes by binding direct repeats located in the promoter regions. One aspect that is unique to the AIP/Agr system is the fact that an AIP produced by one strain of *Staphylococcus* will interfere with another strain's Agr system. This dual role as activator and inhibitor is related to the interaction between the AIP and AgrC. The cyclic structure of AIP is required for interaction with AgrC, but it is the N-terminal tail that is responsible for AgrC activation. Removal of this tail, in fact, results in a universal inhibitor that binds to AgrC but is unable to activate the Agr system (Lyon and others 2000). The Agr system has been linked to pathogenesis in many Gram-positive bacteria. AIP-directed regulation of genes by AgrA results in the production and release of numerous toxins by *S. aureus*, such as alpha-, beta-, and delta-hemolysins, serine proteases (SprE), and toxic shock syndrome toxin 1 (TSST-1) (Parker and Sperandio 2009). *Enterococcus faecalis* is another Gram-positive cell that benefits from the use of AIP signal sensing. This organism uses a 2-component system homologous to the Agr system of *Staphylococcus* to detect the presence of AIP. When AIP is detected, the cell produces and releases 2 extracellular proteases, gelatinase and SprE (Qin and others 2000).

Biofilms in Various Food Industries

Biofilms, or in more conventional terms biofouling, are matrix-enclosed bacterial populations adhering to each other or to surfaces or interfaces (Costerton and others 1995). The biofilm community exhibits primitive homeostasis, a primitive circulatory system, genetic material exchange, and metabolic cooperation (Lappin-Scott and others 1992; Costerton and others 1994, 1995). The presence of biofilms is highly prevalent and difficult to completely eliminate. Biofilms can exist on all types of surfaces in food processing plants ranging from plastic, glass, metal, and wood. The presence of biofilms or attached cells on foods and food contact surfaces often adversely affects food safety, especially in minimally processed foods and raw foods (Frank 2001). Elimination of attached pathogens and spoilage microorganisms on minimally processed produces can be difficult because of the hydrophobic cutin, diverse surface morphologies, and abrasions in the epidermis of fruits and vegetables (Burnett and Beuchat 2001). The foodborne pathogens *E. coli* O157:H7, *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Campylobacter jejuni* are known to form biofilms on food surfaces and food contact equipment, leading to serious health problems, and economic losses (Kumar and Anand 1998). Results from a study of *E. coli* O157:H7 attachment on apples showed that the organism attaches to internal core structures or within tissues of apples and damaged tissues causing its spoilage (Burnett and others 2000). In another study (Takeuchi and Frank 2000), it was reported that *E. coli* O157:H7 prefers to attach to the cut edges of iceberg lettuce. Pseudomonads, the ubiquitous spoilage organisms are also biofilm formers. They are found in food processing environments including drains and floors, on fruits, vegetables, meat surfaces, and in low-acid dairy products (Brocklehurst and others 1987; Piette and Idziak 1991; Criado and others 1994; Hood and Zottola 1997). They produce copious amounts of exopolysaccharides and attach and form biofilms on stainless steel surfaces (Barnes and others 1999) and coexist within biofilms with *Listeria*, *Salmonella*, and other pathogens (Jeong and Frank 1994; Fatemi and Frank 1999; Bagge and others 2001). *Bacillus* is found throughout dairy processing plants (Oosthuizen and others 2001). *Bacillus* and other thermoduric bacteria form biofilms when hot fluids continuously flow over a surface (Frank 2001). Spores and vegetative *B. cereus* cells present in food products can attach to processing equipment and form biofilms. *Salmonella* can be isolated from poultry processing equipment, especially in the slaughter and evisceration area (Helke and Wong 1994; Joseph and others 2001). The poultry processing operation is a wet environment and therefore ideal for biofilm formation. Joseph and others (2001) have shown that *Salmonella* attach and form biofilms in food processing plants. Different species of microorganisms may possess diverse abilities to attach or form biofilms on different surfaces. A study on the attachment of *L. monocytogenes*, *E. coli* O157:H7, and

Pseudomonas fluorescens on iceburg lettuce showed that *L. monocytogenes* and *E. coli* O157:H7 attached preferentially to cut edges, while *P. fluorescens* attached to intact surfaces (Takeuchi and Frank 2000).

Cells embedded in a biofilm are more resistant to cleaning agents and other antimicrobial substances, making them difficult to eradicate from processing equipment (Stoodley and others 2002). Biofilms on processing equipment surface are a source of contamination for food products due to the continuous detachment of cells and spores from the biofilm. Moreover, biofilm formation may cause economic losses due to equipment failure or necessary extensive cleanup (Kumar and Anand 1998). New biofilm control strategies derived from a better understanding of how bacteria attach, grow, and detach are urgently needed by the food industry.

Quorum Sensing and Biofilm Formation

Quorum sensing is thought to play a role in the production of healthy and fully developed biofilms. The complex and multilayer structures of the defined architecture of the biofilm help the bacterial communities to live in a sessile and protected environment (O'Toole and others 2000; Greenberg 2003; Oggioni and others 2006). The formation of biofilms is a multistep process beginning with microbial surface attachment, cell-to-cell aggregation and proliferation, exopolysaccharide matrix production, growth, maturation, and, finally, biofilm detachment or degradation (Camara and others 2002; Yarwood and Schlievert 2003). Quorum-sensing systems appear to be involved in all phases of biofilm formation. They regulate the population density and the metabolic activity within the mature biofilm to fit the nutritional demands and resources available. Bacteria residing within biofilms have markedly different transcriptional programs from free-living planktonic bacteria of the same strain (Asad and Opal 2008).

Quorum-sensing networks also appear to be instrumental in the release of bacteria from the extracellular matrix of the biofilm. The protected sanctuary provided by biofilms presents a problem of escape from the extracellular matrix in which the bacteria reside. When population densities in biofilms become high, bacteria are released into the environment. It is suggested that staphylococci use AI-2 signals to reduce the production of polysaccharide intercellular adhesin and permit the bacteria to escape the biofilm. Short peptides with detergent qualities that are under quorum-sensing control are also deployed to release bacteria for biofilms, when cell densities are high. Sessile bacteria in biofilms provide a continuous source of planktonic bacteria as bacterial populations expand (Novick 2003).

There are several studies that have linked quorum sensing to biofilm formation in food-related bacteria. *Hafnia alvei* isolated from dairy, meat, and fish products is a common bacterial food contaminant. *Hafnia alvei* has the potential to form biofilms (Vivas and others 2008). In a study by Viana and others (2009), AHL molecules were detected in the biofilms of *H. alvei* 071. The *H. alvei* 071 *halI* mutant was deficient in biofilm formation. It was inferred that quorum sensing was required for the differentiation of individual cells of *H. alvei* 071 into complex multicellular structures for biofilm formation. In *Vibrio cholerae* and *Serratia liquefaciens*, the signaling molecules have been observed to control exopolymeric substances (EPS) production and control cell aggregation required for biofilm formation, respectively (González Barrios and others 2006). However, van Houdt and others (2004) found no correlation between biofilm formation and the production of quorum signals in Gram-negative bacteria isolated from food processing environments. But, it was observed that production of quorum signals triggered other biofilm-related responses such as increased resistance to antimicrobials. Though signaling molecules have been detected in biofilms, yet their precise role in biofilm formation is still not clear. Further studies need to be carried out to understand the role played by quorum-sensing signals in different stages of biofilm formation. Biofilms are a persistent problem in food processing environment and inhibiting quorum sensing may eliminate biofilm formation and thus, retard spoilage and benefit food production and safety (Annous and others 2009).

Quorum-Sensing Signals in Food Spoilage

In recent years, there has been an increasing interest in the influence of quorum-sensing signaling molecules related to food spoilage. Various signaling compounds, such as AI-1 and AI-2, have been detected in different food systems such as milk, meat, and vegetables (Bruhn and others 2004; Liu and others 2006; Pinto and others 2007).

Milk and dairy products are easily susceptible to spoilage by psychrotrophic bacteria such as pseudomonads. These Gram-negative bacteria produce extracellular proteinases, lipases, lecithinases, and glycosidases (Dong and others 2000; Stepaniak 2004), and the Gram-positive psychrotrophic aerobic *Bacillus* spp. produce phospholipases that are responsible for the spoilage of some dairy products (Stepaniak 2004). In *Serratia proteamaculans* strainB5a, the production of extracellular lipolytic and proteolytic enzymes is under the regulon of the AHL-based quorum-sensing system, which implies the involvement of quorum sensing in the spoilage of milk by *Serratia* spp. In an experiment by Christensen and others (2003), the inoculation of pasteurized milk with wild-type *S. proteamaculans* caused spoilage after 18 h of storage at room temperature, while the inoculation with a mutant having an inactivated *sprI* gene did not result in spoilage. However, the addition of 3-oxo-C6-HSL to milk inoculated with the sprI mutant caused its spoilage, implying the role of signaling molecules in the spoilage of milk. Similarly, the production of AHLs in both raw and pasteurized milk by the psychrotrophic bacteria *Pseudomonas* spp., *Serratia* spp., *Enterobacter* spp., and *H. alvei* shows that quorum sensing may play a role in the spoilage of milk and dairy products (Whitfield and others 2000; Pinto and others 2007). Moreover, the detection of furanosyl BAI-2 signals in significant amounts in regular milk containing a low bacterial population (102 CFU/mL) suggests the possible involvement of interspecies communication in milk spoilage (Lu and others 2004).

Pseudomonas spp. are responsible for the spoilage of meat and meat products stored under aerobic chill (3 to 8 °C) conditions. Jay and others (2003) have reported the involvement of quorum sensing in the spoilage process of fresh meat products stored under aerobic refrigerated conditions and in the slime formed on the meat surfaces. AHL signals, such as C4-HSL, 3-oxo-C6-HSL, C6-HSL, C8-HSL, and C12-HSL, have been detected in aerobically chill-stored ground beef and chicken by the members of *Pseudomonadaceae* (10^8 to 10^9 CFU/g) and *Enterobacteriaceae* (10^3 to 10^4 CFU/g) (Liu and others 2006). *Hafnia alvei* and *Serratia* spp. have been identified as the dominating species among the AHL-producing *Enterobacteriaceae* isolated from vacuum-packed meat, while *Pseudomonas* isolates have not produced detectable numbers of AHL molecules (Ravn and others 2003). In a study by Lu and others (2004), it was reported that, although the indigenous bacterial population loads were high (6.4 to 8 log CFU/mL), very low levels of BAI-2 activity (less than onefold induction of luminescence compared with the negative control) was detected in beef steak, beef patties, chicken breast, and turkey patties. It has

been suggested that the ground beef-derived fatty acids cause the partial or complete inhibition of AI-2 activity (Soni and others 2008). The cell-free meat extract from spoiled minced pork meat stored aerobically at refrigerated temperatures contained AHLs and BAI-2 signals, and the amounts of these signals were higher at 5 °C than at 20 °C (Ammor and others 2008). Nychas and others (2007) have demonstrated that the addition of cell-free meat extract to an 18-h culture of *P. fluorescens* and *S. marcescens* from fresh meat increased the lag phase of *P. fluorescens* and also the metabolic activity of both the tested strains. Such an increase in metabolic activity has been linked to the presence of some compounds in cell-free meat extract, including the quorum-sensing molecules (Nychas and others 2007). The AHLs have been detected in fresh ground pork meat stored under a modified atmosphere at 5, 10, 15, and 20 °C. AHL production was at its maximum at 10 and 15 °C beginning at 7 and 2 d of storage, respectively, and was associated with the growth of the members of *Enterobacteriaceae* and *Pseudomonadaceae* (Blana and others 2007).

Shewenella putrefaciens and *Pseudomonas* spp. are the specific spoilage organisms of iced marine fish and iced freshwater fish, respectively (Gram and others 2002). AHLs have been detected in a variety of different spoiled commercial fish products, such as cold-smoked salmon, fish fillets, and minced fish. In vacuum-packaged cold-smoked salmon, spoilage is due to interactions of *Enterobacteriaceae* and lactic acid bacteria, *Carnobacterium* sp., and/or *Lactobacillus* sp., growing to high concentration of 10^7 to 10^9 CFU/g (Jorgensen and others 2000). Gram and others (1999) have shown that these food spoilage bacteria can produce AHLs even when they are present at a lower concentration in the food substrate. The AHLs (mainly 3-oxo-C6-HSL) have been produced by the inoculated (under conditions that simulated food environments of 5 °C, reduced oxygen, and 4% NaCl) and indigenous members of the *Enterobacteriaceae* at lower concentrations of 10^6 CFU/mL and 10^5 to 10^6 CFU/g, respectively (Gram and others 1999). A recent study by Connell (2010) has showed that quorum sensing is modulated in bacteria within low-cell-number/high-density bacterial clusters and is also affected by population size and the surrounding medium. This study shows that quorum sensing can be activated even at much lower concentrations as in food-related situations. The detection of 3-hydroxy-C8-HSL known to regulate chitinase activity in the nonbioluminescent *Photobacterium phosphoreum* and *Aeromonas* spp. strains isolated from packed cod fillets implies the possible role of an AHL-based system in the spoilage of crustaceans (Flodgaard and others 2005). The AHLs 3-oxo-C6-HSL, C6-HSL, C8-HSL, and C12-HSL have been detected in *H. alvei*, *S. liquefaciens*, *P. fluorescens*, and *Pseudomonas putida* responsible for the proteolytic activity and spoilage of rainbow trout fillets. Similar AHL-modulated proteolytic activity has been reported in *S. proteamaculans* B5a, a strain originally isolated from cold-smoked salmon (Christensen and others 2003) suggesting the involvement of quorum-sensing signals AHLs in food spoilage.

The pectinolytic activity of *Pseudomonadaceae* or *Enterobacteriaceae* (mostly *Erwinia* spp.) growing to high-cell densities (10^8 to 10^9 CFU g/L) in fruits and vegetables causes enzymatic browning, off-tastes, off-odors, and/or texture breakdown resulting in their spoilage (Liao 1989). *Erwinia* and *Pseudomonas* produce various pectinolytic enzymes, namely, pectin lyases, pectate lyase, polygalacturonase, and pectin methyl esterases, which are responsible for the spoilage of ready-to-eat vegetables, also produce a broad range of AHLs (mainly 3-oxo-C6-HSL and C6-HSL) (Rasch and others 2005). This suggests the involvement of AHL-based quorum-sensing systems in the spoilage of vegetables and fruits. Moreover, inoculation of bean sprouts with AHL-producing pectinolytic *Pectobacterium carotovorum* increased the rate of its spoilage (Rasch and others 2005). In *P. carotovorum* subsp. *Carotovorum*, the production of extracellular enzymes pectate lyase, pectin lyase, polygalacturonase, cellulase, and protease is regulated by 3-oxo-C6-HSL-dependent quorum sensing (Pirhonen and others 1993). These data provide evidence that the bacterial spoilage of some food products is influenced by quorum-sensing-regulated phenotypes. In *Serratia plymouthica* RVH1, a strain isolated from a raw vegetable processing line, a LuxI homolog, SplI, controls the production of the 3 AHLs, C4-HSL, C6-HSL, and 3-oxo-C6-HSL, whose inactivation resulted in the complete loss of 3-oxo-C6-HSL production and decrease in C4-HSL and C6-HSL production. SplI-dependent quorum sensing is involved in the production of extracellular chitinase, nuclease, protease, and an antibacterial compound and 2,3-butanediol fermentation (van Houdt and others 2006). The production of these exoenzymes in RVH1 was reduced in the *splI* mutant and was restored only by the addition of C6-HSL or 3-oxo-C6-HSL (van Houdt and others 2007). In the cucumber rot-associated *S. marcescens* strain MG1 and in *Serratia* sp. ATCC 39006 the secretion of extracellular enzymes involved in spoilage of vegetables is regulated by the SwrI/SwrR quorum-sensing system and its cognate C4-HSL and SmaI/SmaR QS system and its cognate C4-HSL and C6-HSL, respectively (Thomson and others 2000; Riedel and others 2001; van Houdt and others 2006).

Thus, from the above studies, it is clear that AHL-based quorum-sensing systems in Gram-negative bacteria are extensively associated with food spoilage. In table 2, food spoilage of various food products influenced by quorum-sensing-regulated traits is listed. However, there is scarce information on AI-2 and none about the AIPs produced by Gram-positive bacteria in food spoilage. Moreover, the presence of quorum-sensing signaling compounds in pasteurized milk and in meat and fish products that are refrigerated, vacuum-packaged, and under modified atmosphere clearly reveals that the existing preservative techniques are inadequate. Knowledge of the types of spoilage microbes and their quorum-sensing systems in the various categories of food products will help in developing quorum-sensing inhibitors that can then be used as novel food preservatives.

Detection of Quorum-Sensing Signals in Food Using Biosensors

Quorum-sensing signaling molecules can be detected from cell-free supernatants, extracts of food samples, and spent culture supernatants of bacteria isolated from food products (Ammor and others 2008).

AHLs have been identified and quantified and their structures elucidated using mass spectrometry (MS), high-performance liquid chromatography-MS, gas chromatography-MS, and nuclear magnetic resonance spectroscopy (Throup and others 1995; Schaefer and others 2000; Cataldi and others 2007). However, the use of bacterial biosensors has made the detection and quantification of different types of AHLs easier, economical, and faster. The biosensors contain a functional LuxR family protein cloned with a cognate target promoter (usually the promoter of the cognate *luxI* synthase), which upregulates the expression of a reporter gene encoding for a phenotypic response only in the presence of exogenous AHLs, as they do not produce the signaling molecules but only possess their cognate receptors (Steindler and Venturi 2007). Using the reporter strains, the AHLs can be detected either on agar or in the spent culture supernatant. The investigated strain is

Table 2–Bacterial food spoilage influenced by quorum-sensing-regulated phenotypes.

Organism	Food product	Signal-dependent phenotype	Signaling molecules	References
Pseudomonas fluorescens 395	Milk	Proteolytic milk spoilage	C4-HSL and 3OC8-HSL	Liu and others (2007)
Serratia proteomaculans strainB5a	Milk	Lipolytic and proteolytic milk spoilage	3-oxo-C6- HSL	Christensen and others (2003)
Pseudomonas fluorescens	Milk	Proteolytic milk spoilage	L-HSL α-amino-γ-butyrolactones	Dunstall and others (2005)
Pseudomonas phosphoreum and *Aeromonas* spp.	Cod fillets	Chitinolytic activity	3-hydroxy-C8-HSL	Flodgaard and others (2005)
Erwinia carotovora	Vegetables	Cellulolytic and proteolytic spoilage	3-oxo-C6- HSL	Jones and others (1993)
Pectobacterium sp. A2JM	Bean sprouts	Pectinolytic and proteolytic spoilage	3-oxo-C6- HSL	Rasch and others (2005)
Serratia plymuthica RVH1	Vegetables	Chitinase and protease activity	3-oxo-C6- HSL and C6-HSL	Van Houdt and others (2007)
Hafnia alvei and *Serratia* spp.	Vaccum packed meat	Proteolytic spoilage	N-3-oxohexanoyl HSL	Bruhn and others (2004)
Pseudomonas spp.	Meat	Biofilm formation and proteolytic spoilage	AHLs	Jay and others (2003)
Photobacterium phosphoreum and *Aeromonas* spp.	Cod fillets	Chitinolytic spoilage	3-hydroxy-C8-HSL	Flodgaard and others (2005)

cross-streaked close to the biosensor on an appropriate agar medium. The production of AHLs by the test strain will activate the expression of the reporter gene encoding for the phenotypic response. This results in a gradient of responses that can be observed at the meeting point of the 2 strains (Steindler and Venturi 2007).

The AHLs from spent supernatants of late exponential phase cultures can be extracted using the solvents dichloromethane, ethyl acetate, or chloroform. The bacterial extracts are separated by thin-layer chromatography (TLC) on C_{18} reversed phase plates, and then inoculated with the biomonitor systems. The biosensing of exogenous AHL results in the formation of a spot, which is identified according to the AHL standards included in the assay (McClean and others 1997; Schaefer and others 2000; Turovskiy and others 2007) and further detected using analytical techniques (Shaw and others 1997).

Burmolle and others (2003) developed an approach for detecting AHL production using a whole-cell biosensor. The regulatory region of the lux-operon from *Vibrio fischeri* was fused to green fluorescent protein (gfp) resulting in a luxR-PluxI-gfpmut3*-fusion in the high copy plasmid, pAHL-GFP. EC MC4100 harboring the pAHL-GFP responded to the AHL-compound N-octanoyl homoserine lactone (OHL) by expressing green fluorescence. The induced cells were then verified by single-cell analysis using flow cytometry. Using this biosensor, OHL concentrations between 0.5 and 50 nmol/g soil were detected in soil samples. When AHL-producing *S. liquefaciens* was introduced to soil microcosms, expression of gfp was induced in EC MC4100/pAHL-GFP. Thereby, the ability of this strain to detect excretion of AHLs by *S. liquefaciens* in sterile soil was shown. Thus, the biosensor strains containing the genes encoding GFP can be used in the T-streak and TLC assays, and epifluorescent microscopes can be used to detect the GFP emission. These biosensors can also be combined with a flow cytometer for the detection of AHLs.

The bioassays recognize only known compounds and require the use of many reporters for different kinds of AHL molecules. The reporter strains display a high specificity toward the cognate AHL and to a lesser extent to closely related ones. Thus, the detection of a wide range of AHLs requires the use of several biosensors, each responding to AHLs with different structural features.

There are some reporter strains that lack specificity. The TraR of *A. tumefaciens*, sensitive to most 3-oxo-HSLs (Shaw and others 1997) and the *V. fischeri* LuxR-based reporter plasmids such as pSB403, activated by AHLs with carbon chains of C6 or C8 with or without 3-oxo substitutions (Winson and others 1998) are examples of reporter strains lacking in specificity. Using these biosensors can lead to false-positive and false-negative reactions. Moreover, the screened bacterium might produce non-AHL compounds that could potentially interfere or activate the response of biosensors (Holden and others 1999). As different LuxR homologues have diverse affinities with different AHLs, it is not accurate to compare the intensity of a response obtained with different AHLs. Therefore, synthetic AHL must be used to determine the minimal amount of AHL required for a response and the amount necessary for a saturated response to plot the linear dose-response curve (Steindler and Venturi 2007). The high degree of diversity among LuxI and LuxR homologue proteins has become a limiting factor for the development of molecular methods for their easy identification (Whitehead and others 2001).

AI-2 is detected using the biosensor *V. harveyi* BB170 *lux*N::Tn5 that synthesizes all 3 autoinducers, HAI-1 (3OHC4-HSL), an AHL; AI-2, a furanosyl-borate-diester; and Cholerae Autoinducer-1 [CAI-1, an (S)-3-hydroxytridecan-4-one], of unknown structure but senses only AI-2 (Henke and Bassler 2004). BAI-2 activity is calculated as the ratio of luminescence of the test sample to that of the control (negative) sample and expressed as relative activity. The instability of the AI-2 molecule makes the application of chemical methods difficult. Thus, bioassay seems to be the only mode of AI-2 detection. The growth and ability of the biosensor to detect BAI-2 is influenced by the components of the culture medium and the components and additives in food sometimes leading to false-negative or false-positive results (Lu and others 2004). For example, the luciferase in *V. harveyi* is permanently repressed by glucose through catabolite repression, and despite the presence of the *lux*S gene, no BAI-2 activity can be detected. Low pH also affects bioluminescence, making it necessary to adjust the pH of culture supernatants to 7.0 before bioassay testing (de Keersmaecker and Vanderleyden 2003). The effect of all these factors makes the BAI-2 bioassay only qualitative in nature (Turovskiy and Chikindas 2006). Currently, the quantification of BAI-2 is carried out using a LuxP- fluorescence resonance energy transfer (FRET) based reporter strain. The sensor is based on ligand binding-induced changes in FRET between a cyan variant and yellow variant of GFP fused to the termini of the BAI-2 receptor, LuxP (Rajamani and others 2007).

The AI-3 molecule is detected by the β-galactosidase assay using the *E. coli* O157:H7 TEVS232 strain (Sperandio and others 2000) in which the *LEE1* regulatory regions are fused to the *lacZ* reporter gene in TE2680. TEVS232 is grown in fresh medium or in medium supplemented with *Pseudomonas cepacia*. Cultures are diluted 1:10 in Z buffer, and β-galactosidase activity is measured by using *o*-nitrophenyl β-D-galactopyranoside as a substrate (Walters and others 2006).

Methods for the detection and quantification of AIPs mainly involve the application of nisin. The bioassays based on nisin-inducible bioluminescence (Wahlstrom and Saris 1999; Immonen and Karp 2007) and fluorescence (Reunanen and Saris 2003; Hakovirta and others 2006) involve reporter genes placed under the control of a nisin-inducible promoter, either bacterial luciferase genes (*lux*) or the GFP gene (*gfp*). The GFP-based nisin microplate bioassay uses an *L. lactis* strain, LAC275, which chromosomally encodes the nisin regulatory proteins NisK and NisR and a plasmid with a GFP variant gene, gfp_{uv}, under the control of the nisin-inducible *nisA* promoter. This strain is capable of transducing the signal from extracellular nisin into measurable GFPuv fluorescence through the NisRK signal transduction system. The sensitivity of this biosensor is quite high and it can detect nisin concentrations upto of 10 pg/mL in culture supernatant, 0.2 ng/mL in milk, 3.6 ng/g in processed cheese, 1 ng/g in salad dressings and crushed canned tomatoes, and 2 ng/g in liquid egg (Hakovirta and others 2006). However, autofluorescence of the cellular background lowers the sensitivity (Hakkila and others 2002). Before measuring the fluorescence, supernatant must be removed as it absorbs light at the same wavelengths as GFP (Immonen and Karp 2007).

2-Alkyl-4-quinolones (AHQs) and 2-heptyl-4-quinolone of the *Pseudomonas* spp. are detected and quantified using the *lux*-based *P. aeruginosa* AHQ biosensor strain (Fletcher and others 2007). In this method, the reporter strain is used either in TLC or microtiter plate assays, wherein the bioluminescence or the green color of pyocyanin is detected as end points. In the TLC assay, the bacterial extracts or cell free supernatant are separated on TLC plates. The dried TLC plates are overlaid with the AHQ biosensor. AHQs if present are seen as luminescent green spots. In the microtiter assay, bacterial extracts or CFS are added to a growth medium containing the AHQ biosensor and the resulting light is proportional to the AHQ content of the sample.

Quorum-Sensing Inhibitors in Food Preservation

The involvement of AHLs in the regulation of virulence factors in several opportunistic human and plant pathogenic bacteria has paved the way for an intense search of compounds that can specifically block AHL communication. In the clinical scenario, it is envisioned that such compounds can block expression of virulence without affecting growth and also decrease the risk of resistance development. Quorum-sensing inhibitors are typically analogues of the AHLs (Eberhard and others 1986), or compounds that degrade AHLs (Dong and others 2000). A promising group of quorum-sensing inhibitors is the halogenated furanones produced by the Australian red alga, *Delisea pulchra* (Givskov and others 1996). These interfere with the receptor proteins and release the AHL signal (Manefield and others 1999, 2002). Treatment with these compounds has reduced the expression of AHL-regulated virulence factors and biofilm formation in *P. aeruginosa* and *S. liquefaciens* (Givskov and others 1996; Rasmussen and others 2000; Hentzer and others 2002). The cytotoxicity and chemically unstable nature of these halogenated furanones has prompted the screening of nontoxic quorum-sensing inhibitors from natural sources. Plants used in traditional medicine are one of the most promising areas in the search for new biologically active compounds (Hullati and Rai 2004; Jamuna Bai and others 2011). In a study by Vattem and others (2007), dietary phytochemicals from plants known to have several health benefits and antimicrobial activity, exhibited quorum-sensing inhibitory activity at sublethal concentrations. The bioactive dietary phytochemical extracts from common dietary fruit, herb, and spice extracts significantly inhibited quorum sensing in *Chromobacterium violaceum* O26 (CVO26) and CV 31532. The extracts also inhibited swarming motility in pathogens EC O157:H7 and *P. aeruginosa* (PA-01), known to be modulated by quorum sensing. Vanilla, a widely used spice and flavor, can inhibit bacterial quorum sensing. The extract of vanilla beans in the *C. violaceum* CV026 bioassay effectively inhibited bacterial quorum sensing. Vanilla extract, or specific compounds isolated from it, may be used as novel quorum-sensing inhibitor. These will have a greater advantage for human use than the toxic furanone compounds, as vanilla has been used safely for a long time (Choo and others 2006). Girennavar and others (2008) have reported that natural furocoumarins from grapefruit juice act as the potent inhibitors of AI-1 and AI-2 activity and biofilm formation in *S.* Typhimurium, EC, and *P. aeruginosa*. These results suggest that grapefruit juice can serve as a safe source of alternatives to the halogenated furanones to develop strategies targeted at microbial quorum sensing. Thus, quorum-sensing inhibitors can also be used as food preservatives in selected foods where AHL-regulated traits are responsible for food spoilage. The finding of quorum-sensing inhibitory compounds from plants raises the possibility of identifying active quorum-sensing inhibitory compounds from a plethora of natural sources. Quorum-sensing inhibitors may prevent colonization of food surfaces, toxin formation, and proliferation of food-related bacteria. The natural occurrence of quorum-sensing inhibitors is an important consideration for the assessment of their toxicological status and may facilitate their use in food as preservatives and hence pave way for the application of novel food preservation techniques.

Conclusion

Food spoilage, a consequence of intrinsic biochemical changes and microbial activities, is a complex process. Recent studies have provided evidence that quorum sensing, which was earlier implicated in microbial virulence and pathogenesis, may also play a role in food spoilage. AHL- and AI-2-based quorum-sensing systems associated with Gram-negative bacteria in different food ecosystems have been detected. Further studies have to be carried out to know if AIPs of Gram-positive bacteria have a potential role in food spoilage. Although quorum-sensing signaling molecules have been detected in spoiled foods, the exact role played by them in food spoilage is still not clear. Understanding the mechanism of quorum-sensing systems in food ecosystems can help in targeting quorum sensing and addressing the problem of food spoilage. Quorum-sensing inhibitors could be used as food preservatives to enhance food safety and increase shelf life.

Acknowledgments

The authors gratefully acknowledge Dr. K.S. Manja, Former Director, Defence Research and Development Organization, for critically reviewing and providing constructive suggestions for improving the manuscript.

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