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MINIREVIEW

Quorum Sensing in the Context of Food Microbiology

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Food spoilage may be defined as a process that renders a product undesirable or unacceptable for consumption and is the outcome of the biochemical activity of a microbial community that eventually dominates according to the prevailing ecological determinants. Although limited information are reported, this activity has been attributed to quorum sensing (QS). Consequently, the potential role of cell-to-cell communication in food spoilage and food safety should be more extensively elucidated. Such information would be helpful in designing approaches for manipulating these communication systems, thereby reducing or preventing, for instance, spoilage reactions or even controlling the expression of virulence factors. Due to the many reports in the literature on the fundamental features of QS, e.g., chemistry and definitions of QS compounds, in this minireview, we only allude to the types and chemistry of QS signaling molecules *per se* and to the (bioassay-based) methods of their detection and quantification, avoiding extensive documentation. Conversely, we attempt to provide insights into (i) the role of QS in food spoilage, (ii) the factors that may quench the activity of QS in foods and review the potential QS inhibitors that might "mislead" the bacterial coordination of spoilage activities and thus may be used as biopreservatives, and (iii) the future experimental approaches that need to be undertaken in order to explore the "gray" or "black" areas of QS, increase our understanding of how QS affects microbial behavior in foods, and assist in finding answers as to how we can exploit QS for the benefit of food preservation and food safety.

In the last few decades, our perception of bacteria and their communities has changed dramatically. Bacteria have most often been considered populations of cells that act individually, but it is now increasingly apparent that there is much interaction and communication among adjacent cells (55).

BACTERIAL COMMUNICATION

Quorum sensing (QS), a term introduced by Fuqua and Winans (32) to describe cell-to-cell communication, is the mechanism used by bacteria to understand changes in their environment and consequently to apply specific strategies that allow adaptation to environmental stress in space and time. This continuous adaptation process may be affected by microbial communication (136, 140). Indeed, strategies such as enhanced access to nutrients or environmental niches, mounting defensive responses against eukaryotic hosts and competing organisms (i.e., secretion of virulence factors), optimization of the ability of the cell to differentiate into morphological forms (i.e., biofilm formation, sporulation) and adaptation/survival in hostile, growth-restrictive environments are some bacterial behaviors dictated by the use of signal-response systems (4, 112, 123). In its simplest form, cell-to-cell signaling results from the production of small, diffusible signal molecules called autoinducers. The signal molecules are secreted at a basal level during bacterial growth by emitter cells and accumulated in the surrounding environment. This environment dictates the fate of the quorum molecule, for instance, the rate of its accumulation to a threshold concentration, which then triggers a contextually appropriate genetic program. The concentration of these signaling compounds in the environmental (e.g., growth) medium or matrix creates zones of reduced concentration, i.e., gradient concentration across the cell/colony/environment interface. However, limited diffusion of these compounds between cells leads to locally high accumulation internally. When this concentration reaches the aforementioned threshold level (i.e., the quorum level), the signaling molecules bind to receptors on or in the bacterial cell, leading to changes in gene expression in the responding cell. For intraspecies QS, the emitter and the responder are usually the same cells. Often, but not always, the genes that are involved in the synthesis and response activate their own expression—explaining the term autoinducer, e.g., the phenomenon occurs without any external intervention (81). It should be noted that a signaling molecule is considered such since it acts at low concentrations and is not involved in primary metabolism (55).

In general, QS is omnipresent in many known human and plant bacterial species as well as in extremophiles such as *Natronococcus occultus*, *Halomonas* genus, *Thermotoga maritima*, and *Acidithiobacillus ferrooxidans* (52, 69, 88, 106). With regard to pathogenic Gram-negative bacteria, including the genera *Agrobacterium*, *Brucella*, *Burkholderia*, *Erwinia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia*, *Vibrio*, and *Yersinia*, the QS mechanism for the regulation of virulence factor syntheses has been exploited (132). This mechanism has also been used by *Bacillus*, *Enterococcus*, *Staphylococcus*, *Streptococcus*, *Streptomyces*, and *Rhizobium* genera to develop genetic competence or produce antimicrobial peptides or exotoxins or for biofilm formation and nitrogen fixation (45, 95). Bacteria not only communicate with members of the same species but may also "eavesdrop" on the "conversation" of other species and modulate their behavior in response to signal molecules they do not synthesize (29).

As mentioned above, existing studies have mainly focused on the molecular aspects of cell-to-cell communication, i.e., how QS affects virulence, biofilm formation, sporulation or conjugation,

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etc. Conversely, much less attention has been paid to the ecological context of why bacteria produce signaling molecules and respond to both intra- and interspecies signals, and even less attention has focused on how the ecological niche affects this communication. This is the case with the niches present in food ecosystems, where the role of cell-cell communication has only recently received attention from food microbiologists, despite the fact that a growing body of evidence has been collected suggesting that bacterial food spoilage may also be regulated by QS systems (1, 112). So far, few studies have investigated the influence of food system conditions on autoinducer signal production by foodborne pathogens (9, 17, 70, 79, 80) and the influence of these QS signals on the survival/growth of pathogenic bacteria in foods (9, 115). Soni et al. (116, 117), for example, reported that the survival and virulence expression of a *luxS* mutant strain of *Escherichia coli* O157:H7 was enhanced in the presence of autoinducer-2 (AI-2). Similarly, production of AI-2 by *Salmonella enterica* serovar Typhimurium contributed significantly to its ability to colonize in chicken intestine compared to the LuxS$^-$ mutant strain (9).

Disrupting the QS pathway can play a major role in controlling microbial gene expression related to human infection and food spoilage. The role of QS signaling molecules involved in food spoilage needs to be understood in the effort to block the causative cell-to-cell communication and prevent microbial spoilage. Quorum-sensing inhibitors (QSIs) 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. It is also challenging to understand which factors in foods may influence cell-to-cell signaling and how pathogens respond in the presence of signals produced by other bacteria (114). This could potentially lead to identification of species-specific molecules and/or development of interventions that could be employed to control or inhibit the QS-regulated behaviors of spoilage and pathogens, ultimately impacting food quality and safety.

GROUP OF COMMUNICATION COMPOUNDS

Several classes of signaling molecules of microbial origin have now been identified and can be divided into four broad categories: (i) *N*-acyl homoserine lactones (AHLs), which are fatty acid derivatives generically called autoinducer-1 (AI-1) and are produced and used by gram-negative bacteria mainly for intraspecies communication (113); (ii) a furanosyl borate diester, which is derived from the recycling of *S*-adenosyl-homocysteine to homocysteine also known as autoinducer-2, is produced by both Gram-positive and Gram-negative bacteria, and is thought to serve as a universal signal for interspecies and intraspecies communications (22, 139); (iii) autoinducer-3 (AI-3), which serves as the QS signal for enterohemorrhagic *Escherichia coli* (EHEC) virulence genes and cross talk with the mammalian epinephrine host cell signaling systems (103, 118); and (iv) autoinducing peptides (AIPs), which are produced and used by Gram-positive bacteria (75, 121).

Other molecules similar to those in the QS systems also have been described. The 2-heptyl-3-hydroxy-4-quinolone (PQS) is an intracellular signal molecule of a new type that has been found in *Pseudomonas aeruginosa* (91, 131). In addition to PQS, diketopiperazines (cyclic dipeptides), which are small and diffusible molecules, were found to be involved in QS systems (46). These molecules have biological and pharmacological effects on the cells of multicelled organisms, suggesting their role in bacterial conversation with plant and animal cells rather than with other bacteria. *Vibrio cholerae* autoinducer-1 (CAI-1), the chemical nature of which is unknown, is proposed to be responsible for *Vibrio*-specific signaling (41, 42). In conclusion, the main groups of signal molecules involved in bacterial QS are two; one is the peptide derivatives typically used by Gram-positive bacteria, while the fatty acid derivatives are exploited by the Gram-negative bacteria.

Recently, it was suggested that there is a high level of specificity displayed by LuxR-type proteins for their natural AHLs (33), and this may be one of the reasons why relatively few synthetic AHL-based derivatives capable of exhibiting heightened activities relative to native AHLs have been identified. This is a particular concern for studying their structure-activity relationship (SAR) (33).

INTRASPECIES CELL-TO-CELL COMMUNICATION

A great number of Gram-negative bacteria synthesize multiple AHLs. AHLs are characterized by a homoserine lactone ring that is N-acylated with a fatty acyl group at the C-1 position. The N-acyl chain may vary in length, saturation level, and oxidation state. Typically, the acyl chains range from 4 to 18 carbons, may contain double bonds, and often contain an oxo or hydroxyl substituent at the C-3 position (133). AHLs are synthesized with the reaction of *S*-adenosylmethionine (SAM) (an essential metabolite in the central metabolism) with an acyl-acyl carrier protein, which is typically carried out by an enzyme of the LuxI family of the AHL synthases, and sensed by the response transcriptional regulators of the LuxR family. The LuxR/AHL complex is responsible for up- or downregulation of multiple target genes (123). Bacterial species may synthesize more than one type of AHL, while the same type of AHL may be produced by representatives of different bacterial genera (27, 89, 90, 122). Short-chain AHLs are generally diffusible throughout the bacterial membrane, while long-chain AHLs seem to be actively transported in and out of the cells via efflux and influx systems (133). Several factors may influence the concentration and type (i.e., the length and substitution of the C-3 of the acyl chain) of AHLs, including temperature, pH, NaCl, growth medium, inoculum size, and bacterial growth phase (36, 76, 77, 79, 142).

In Gram-positive bacteria, cell-to-cell communication is accomplished via peptides or modified peptides (autoinducing peptides). AIPs are characterized by a small size (i.e., ranging from 5 to 26 amino acid residues), high stability, specificity, and diversity and can be linear or cyclic (25) These peptides are ribosomally synthesized as precursor peptides, subsequently processed to form the active mature peptide autoinducer signal molecule, and then secreted via an ATP-binding cassette (ABC) transporter. Depending on whether the sensor is on the cell surface or cytoplasm, the peptides can exert their function either intercellularly or extracellularly (25, 120).

INTERSPECIES CELL-TO-CELL COMMUNICATION

The only currently known family of signal molecules shared by more than 70 species of both Gram-negative and Gram-positive bacteria is autoinducer-2 (29). AI-2 signal molecules are considered to be a universal language, because they allow bacteria to respond not only to endogenously produced AI-2 but also to AI-2 produced by other bacterial species in the vicinity. The biosynthetic pathway for AI-2 has been described (101). AI-2 is synthesized in three enzymatic steps from SAM. Following methyl transfer from SAM, *S*-adenosyl-homocysteine (SAH) is formed.

Subsequently, Rfs enzymes remove adenine from SAH to form *S*-ribosyl-homocysteine (SRH). Finally, the LuxS protein cleaves SRH to produce homocysteine (HC) and AI-2 precursor 2,4-dihydroxy-2-methyldihydro-3-furanone (DHMF). The latter cyclizes spontaneously and gives rise to a number of related furanone derivatives. The exact structure of AI-2 furanone has not yet been determined (109). AI-2 production may be influenced by temperature and growth medium (9, 17).

QUORUM SENSING IN THE CONTEXT OF FOOD AND FOOD PROCESSING

Microbial ecology of food contact surfaces. Biofilms are groups of bacteria encased in a self-produced extracellular matrix (19, 20) which allow them to enjoy a number of advantages, such as they are more resistant to antimicrobial agents, cleaning agents, and other antimicrobial substances compared to their planktonic counterparts, making them difficult to be eradicated from processing equipment (35, 51, 119). Additionally, the biofilm community exhibits primitive homeostasis, a primitive circulatory system, genetic material exchange, and metabolic cooperation (18, 19, 60).

Biofilms formed on stainless steel surfaces in food-processing environments are of special importance since they have the potential to act as chronic sources of microbial contamination, leading to food spoilage and transmission of diseases (10, 59). 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 so as to fit the nutritional demands and resources available. Bacteria residing within biofilms have markedly different genome/transcriptional programs from free-living planktonic bacteria of the same strain (3, 34; E. Giaouris, G.-J. Nychas, and P. N. Skandamis, unpublished results).

A growing body of evidence demonstrates that QS contributes to biofilm formation by many different species (21, 37, 40, 73, 97). Recently, Yoon and Sofos (141) showed that biofilm formation by *Salmonella* Thompson on stainless steel, under monoculture conditions (72 h at 25°C), was similar in AI-2-positive and -negative strains. However, taking into account that *Salmonella* possess SdiA, a receptor for AHLs which may be produced by resident flora on food contact surfaces (80, 115), the effect of AHLs on biofilm formation by this pathogen in multispecies environments needs further study. The challenge becomes more intriguing given that microflora on inadequately cleaned and disinfected food industry surfaces is a complex community, unlike the pure-species biofilms studied in the laboratory (10, 96, 112). The interactions between the different species may influence the biofilm-forming capacity of individual strains, and this may be a QS-mediated process (48).

There are several studies that have linked QS to biofilm formation in food-related bacteria. *Hafnia alvei* isolated from dairy, meat, and fish products is a common bacterial food contaminant. *Hafnia alvei* 071 strain has the potential to form biofilms (130), while this was not evident with *H. alvei* 071 *halI* mutant, and it was concluded that QS was required for the differentiation of individual cells of *H. alvei* 071 into complex multicellular structures for biofilm formation. The control of exopolymeric substances (EPS) by AI-2 of *Vibrio cholerae* and *Serratia liquefaciens*, have been observed (37). The production of EPS is required for cell aggregation that leads to biofilm formation. This was not confirmed in Gram-negative bacteria isolated from food-processing environments (127). Though signaling molecules have been detected in biofilms, their precise role in biofilm formation is still not clear. Further studies under controlled *in vitro* conditions, involving the effects of specific QS signals in monoculture or composite cultures on the dynamics and stress response (e.g., resistance to sanitizers) of biofilms need to be carried out to understand the role played by QS signals in different stages of biofilm formation. Biofilms are a persistent problem in food-processing environments, and inhibiting QS may eliminate biofilm formation and thus retard spoilage and benefit food production and safety (2). Potential involvement of QS in regulation of biofilm formation by food-borne pathogens on food contact surfaces could open new research avenues toward our efforts to eliminate these surface-attached communities. This is the case with a recent study of Chorianopoulos et al. (15) who showed that the biofilm development by the pathogen *Salmonella enterica* serovar Enteritidis PT4 on stainless steel (SS), in the presence of various compounds (metabolites) produced by *Hafnia alvei*, a psychrotrophic spoiler microorganism associated with animal-originating foods that incubation of coupons in 50% cell-free supernatant (CFS) resulted in a significant reduction (ca. 1 log CFU cm^{-2}) in the number of strongly attached/biofilm cells in the first 24 h, compared to 0% or 20% CFS. Thin-layer chromatography revealed the existence of signaling compounds, in the form of acylhomoserine lactones, in the two growth media containing CFS (20 and 50%), during the entire incubation period. However, the exogenous addition in pure BHI broth of various commercial synthetic AHLs did not significantly influence the early stages of *Salmonella* biofilm formation.

Food microbial ecology. The food matrix should be considered in those environments where quorum or other sensing molecules are released but do not have consistent diffusion or chemical characteristics. The importance of the external environment in altering sensing signals has started to be appreciated (47). Indeed, sensing processes are now known to be influenced by environmental parameters, including temperature, ligand concentration, pH, and water and oxygen availability (107).

The role of QS in food microbial ecology has only recently been investigated, and available data are rather limited. In most of the available studies, various signaling compounds such as AI-1 and AI-2 have been reported to be present and/or increase their concentration in different food systems (e.g., milk, meat, and vegetables) (1, 5, 11, 60, 65–67, 70, 93, 123). Although the production of these compounds has been attributed to certain members of the food microbial association, e.g., pseudomonads, members of the *Enterobacteriaceae* family, and lactic acid bacteria (LAB), very little is known about the influence of food processing and storage conditions (e.g., temperature, packaging) on the qualitative and quantitative release of these signals in foods. The dominant organisms in a food ecosystem at different stages of storage vary depending on product type, its intrinsic properties, and the (extrinsic) conditions surrounding the product (49, 85, 92). In fact, the dominance of organisms is the result of a microbial succession with certain organisms being able to have implicit properties or develop specific strategies, which allow them to acquire numeric superiority in the niches that develop from the interplay of the physicochemical properties of the food and storage conditions in space and time (7, 8, 138). Microbial association, specific spoilage organisms (SSOs), or ephemeral spoilage organisms (ESOs) are terms that have been used to describe the fact that only a small

fraction of microorganisms temporally dominate their succession in a food ecosystem at the time of spoilage (7, 85).

It should be noted that in the majority of foods, the *in situ* environment will mean association of microbial cells with a solid substrate either through entrapment, constrained growth, or attachment or a combination thereof. As a result, the cells are immobilized and localized in high densities and may grow as microcolonies or biofilms (23, 54, 137). At different sites within the food, there may be variation in the levels of oxygen, pH, water activity, nutrients, and in certain foods, preservatives. This results in a series of interconnected microenvironments, some of which may be preferential for microbial growth (138). With the possible exception of highly processed products, foods harbor a variety of microorganisms, which include different species of bacteria and strains within these species. The growth responses and activity of any one species or strain, whether it is an unwanted spoilage or pathogenic microorganism or a desirable biocontrol organism, will, in most cases, be determined by the presence of other species (8) and the *in situ* cell-to-cell ecological interactions, which often occur in the solid phase of foods.

Thus, the food microbial ecological approach is pertinent to the analysis of cell-to-cell communication in different food ecosystems, with for example, the following questions. (i) What is the critical concentration of QS signals needed by microorganisms to recognize the quorum and govern their gene expression profiles? (ii) Is there any diffusion and chemical degradation of the signals due to the (dynamic) abiotic conditions of the food product? (iii) Is the spatial distribution of cells more important than the density of cells in QS signaling in solid food products where microcolonies are formed on the surface or within the food matrix? (iv) Is it possible that other species or strains, which coexist in the same environment with the classical QS producers decompose and/or produce the same autoinducer(s)? (v) Do these QS signaling molecules act in a similar way even if in some cases the SSOs or ESOs are the same (77, 79). Since the confirmation of the presence or absence or the determination of the levels of QS compounds, even when advanced analyses, such as gas chromatography-mass spectrometry (GC-MS) or high-performance liquid chromatography (HPLC)-MS is carried out in foods, does not always answer the key question of how they influence spoilage in foods and how food components are affecting QS, alternative suitable direct or indirect methods for the accurate measurement of the levels and effects of autoinducer compounds should be applied.

These questions should be addressed in a variety of solid and semisolid food systems (e.g., meat, fish, cheese, and vegetables) because these foods are contaminated with a wide range of microbial taxa and represent different abiotic environments. The food structure strongly affects the type (planktonic, colonial, immobilized) and the dynamics of growth and potentially the physiology of bacteria, due to accumulation of metabolic products (e.g., within a colony) compared to diffusion of metabolites away from cells in a liquid culture (57, 76, 125). Thus, considering that growth within a colony results in an inevitable proximity of cells and increase in cell density in a limited space, the release of QS compounds and their rapid diffusion within the colony might be more directly perceptible and thus, have greater impact on immobilized cells or cells within a colony than on cells growing planktonically in a liquid system (57, 76). It should be noted that there are limited studies in which the above queries have been addressed. For example, in a recent study of Dourou et al. (24), it was reported that the growth of 4 different strains of *Salmonella* was affected by the presence of acylated homoserine lactones and autoinducer-2 signaling compounds and/or other novel signals existing in cell-free supernatant, produced by pathogenic and spoilage bacteria, such as *Pseudomonas aeruginosa*, *Yersinia enterocolitica*-like GTE 112, *Serratia proteamaculans* 00612 (16), *Y. enterocolitica* CITY650, and *Y. enterocolitica* CITY844. It was shown that (i) the growth kinetic parameters and the microbial activity of four *Salmonella* strains were affected by the addition of CFS produced by other pathogenic and spoilage bacteria and that (ii) there was not a uniform type of response in the bacterial strains tested, meaning that the effect of AHLs or AI-2 signaling molecules on growth and metabolic activity of the bacterium is rather dependent on the strains producing the signaling compounds in the CFSs.

The suggestion that these QS compounds can also act as probes or proxies, thereby offering an alternative angle to communicating cell density, by providing individual cells with information on the diffusion and flow properties of their environment, preventing the wasteful synthesis of "expensive" extracellular substances, such as exoenzymes, bacteriocins, siderophores, and other effectors (41, 53, 96), could possibly assist in explaining these findings (24). Provided that these substances remain in the (immediate) environment surrounding cells, they may increase nutrient availability and ultimately benefit their producers (104). Indeed, the addition in the reaction cells of QS signaling compounds and/or other potential signals existing in CFS and produced by the tester strains resulted in rapid mixing and diffusion into the microenvironment of pathogens, thereby altering *Salmonella* activity possibly through an over- or underproduction of substances necessary for growth (e.g., enzymes, metabolites, etc.) (104). It needs to be noted, however, that other unknown nonsignaling compounds (e.g., products of proteolysis or of carbohydrate hydrolysis) also present in the CFS of the tester strains might have contributed to the observed phenomenon and should not be ignored. Nonetheless, an extensive GS-MC and HPLC analysis of the tested reaction cells with or without CFS undertaken in a similar study did not reveal any difference in their composition (15).

Although direct extrapolation of such findings to real food ecosystems is currently difficult, it is conceivable that these results may represent various situations of interactions between bacteria and signaling compounds in the microenvironment of foods.

This is the case with the findings of Soni et al. (116, 117) who reported that the presence of AI-2 molecules promoted the survival of *E. coli* O157:H7 cells, whereas the protective effect of AI-2 molecules was negated in the presence of ground beef extracts that contained a significant amount of inhibitory activity.

Microbial spoilage in foods of animal and plant origin. Foods of animal origin are considered to be milk and dairy products, meats and meat products, and fish and seafood products. The spoilage of such foods is mainly associated with the presence of high numbers of Gram-negative proteolytic psychrotrophic bacteria, mainly *Pseudomonas* spp. and genera of the *Enterobacteriaceae* family when these products are stored aerobically, while the contribution of *Brochothrix thermosphacta* and LAB under modified atmospheres is also evident (85). In fact, the concentration of low-molecular-weight compounds (glucose, lactate, free amino acids, etc.) regulate the type and rate of spoilage in these products (84, 85, 87). This is due to the fact that only the depletion of these compounds affects the activity of extracellular proteolytic en-

TABLE 1 Studies of the potential role of QS in spoilage

QS study	QS signal response based on the bioassay performed[a]	Reference(s)
Meats and meat products		
Enterobacteriaceae strains isolated from vacuum-packed chilled meat	C_6-3-oxo-HSL, C_6-HSL	Gram et al. (38, 39)
Meat extracts and isolated *Enterobacteriaceae* strains from chilled stored vacuum-packed meat	C_6-3-oxo-HSL, C_6-HSL	Bruhn et al. (11)
Food samples (e.g., beef, chicken, and turkey products [AI-2-like activity])	Borated AI-2	Lu et al. (70)
Pseudomonad and *Enterobacteriaceae* isolates from aerobically chilled stored proteinaceous raw foods	Medium- and long-side-chain AHLs	Liu et al. (67)
AHL signals during storage of aerobically chilled stored ground beef	C_4-HSL, C_6-3-oxo-HSL, and undefined AHLs	Liu et al. (67)
Aeromonas hydrophila strains isolated from meat	C_4-HSL	Medina-Martinez et al. (78)
AHL production of *Yersinia enterocolitica* strains in fresh beef and pork extracts	C_6-3-oxo-HSL, C_6-HSL	Medina-Martinez et al. (79)
Poultry meat-derived fatty acids as inhibitors of AI-2	Borated AI-2	Widmer et al. (135)
Survival and virulence gene expression of *E. coli* O157:H7 in the presence of AI-2 and ground beef extracts	Borated AI-2	Soni et al. (116, 117)
Ground beef-derived fatty acids as inhibitors of AI-2	Borated AI-2	Soni et al. (116, 117)
Cell extracts from minced pork stored aerobically at 5 and 20°C	Short-, medium-, and long-side-chain AHLs, AI-2	Nychas et al. (86)
Pseudomonas fragi isolated from fresh and spoiled meat	Borated AI-2	Ferrocino et al. (30)
Production of quorum-sensing signals by *E. coli* O157:H7 strain in meat broths (beef and pork)	AI-2 activity	Silagyi et al. (110)
Lactic acid bacteria isolated from minced beef packaged under modified atmospheres	AI-2-like activity	Blana et al. (5, 6)
Vegetables		
Detection of AHL in bean sprouts stored at 5°C	Broad range of AHLs	Gram et al. (39)
AI-2-like activity in fresh foods (tomato and carrot)	AI-2 activity	Lu et al. (70)
Extracts from commercial bean sprouts	C_6-3-oxo-HSL	Rasch et al. (99, 100)
Enterobacteriaceae, pseudomonads, and *Vibrionaceae* strains isolated from commercial bean sprouts	C_6-3-oxo-HSL, C_{10}-3-oxo-HSL, C_{10}-3-hydroxy-HSL, C_4-HSL, C_6-HSL, C_8-HSL, C_{10}-HSL, and undefined AHLs	Rasch et al. (99, 100)
Presence of AI-2-like activity in cell-free supernatants of eggplant, squash, tomato, pepper, cucumber, potato, and carrot	AI-2-like activity	Lu et al. (71)
AHL production of *Yersinia enterocolitica* strains in lettuce and cucumber extracts	C_6-3-oxo-HSL, C_6-HSL	Medina-Martinez et al. (78, 79)
AHL production of *Aeromonas* spp. in a food system of broccoli, parsley, and spinach	C_4-HSL	Medina-Martinez et al. (78, 79)
Production of quorum-sensing signals by *E. coli* O157:H7 strain in spinach broth	AI-2 activity	Silagyi et al. (110)

[a] HSL, homoserine lactone; AHLs, *N*-acyl homoserine lactones.

zymes and thus may influence both the development of microbial community and the habitat and activity domain (i.e., the microbial "domain") (8, 67). On the other hand, the spoilage of vegetables and fruits, which is commonly manifested as visual defects, including enzymatic browning, off-flavor/off-odors and/or texture breakdown is often caused by the pectinolytic activity of members of the *Pseudomonadaceae* or *Enterobacteriaceae* (mostly *Erwinia* spp.) family growing to high cell densities (10^8 to 10^9 CFU g^{-1}) (13, 63, 72). A range of pectinolytic enzymes can be produced microbiologically: pectin lyases, pectate lyase, polygalacturonase, and pectin methyl esterases (64). Therefore, because food spoilage is a phenomenon requiring high levels of microbial populations, QS may be a potential regulatory spoilage mechanism. For this reason, the potential role of QS in spoilage has been investigated, although by a limited number of studies as indicated in Table 1.

PROMOTING VERSUS QUENCHING QUORUM SENSING

Food spoilage is considered to be a process that renders a product undesirable or unacceptable for consumption. This complex ecological phenomenon is the outcome of biochemical activity, through various enzymes of microbial association, which will eventually dominate according to the prevailing ecological determinants on each food system (85). Indeed, a number of microbial extracellular enzymes, e.g., pectate lyase, pectin lyase, polygalacturonase, cellulase, lipases, chitinase, nuclease, and protease, have recognized contribution to food spoilage. Most of these enzymes have been reported to be regulated by QS (16, 31, 53, 66, 68, 94, 101, 105, 126, 128, 129, 134), suggesting that one of the potential means of preventing or delaying food spoilage could be the disruption and/or control of cell-to-cell communication. Alternative strategies could make use of QS compounds in order to generate

false sensing and confuse bacteria, generating a kind of "illusion" in that there are already too many bacteria, and hence, they should cease their growth and/or metabolic activity. Such approaches are usually referred to as quorum quenching (QQ) or QS inhibition (QSI). It should be noted that research on QSI has been focused on food safety, e.g., on regulation of virulence, biofilm formation, or other clinical level issues, whereas reports or evidence on the use of QQ or QSI in food preservation are scarce. The fact that several compounds that block QS without affecting growth of the bacteria have been described in the literature (1) means that the term quenching should be viewed skeptically in the case of spoilage. Indeed, recent studies have provided evidence that the low activity of AI-2 signals found in cell-free meat extract (CFME) in comparison with the activity reported for the prevailing lactic acid bacteria isolates (5, 6) raise questions on the contribution of these compounds: (i) in the selection of specific LAB during the storage of meat and (ii) on the actual role of QSI in food spoilage, given that spoilage finally occurred despite the existence of "indigenous" QSI in the food system (71).

In practice, various QSIs, such as halogenated natural furanones or synthesized derivatives, have been extensively investigated and have been successfully applied to prevent toxin production, minimize bacterial resistance, inhibit expression of virulence factors etc. (74), but data on their use in food preservation are scarce. It should be mentioned, however, that the halogenated furanones currently investigated are chemically reactive and unstable and might be too toxic to be used for the treatment of bacterial infections in humans (44) or may be lethal to some animals such as rainbow trout (98).

Plants including crown vetch, carrot, soybean, water lily, tomato pea seedlings, habanero garlic, bean sprouts, garlic, chamomile, and vanilla and their natural compounds, such as cinnamaldehyde and ascorbic acid, have been found to produce compounds capable of interfering with bacteria (101). For example, garlic extract is reported to contain a minimum of 3 different QS inhibitors, one of which is identified as an acyclic disulfur compound (101). In particular, this QSI exerts a strong antagonistic effect on LUXR-based QS. The antimicrobial action of these plant extracts has been widely used in the food and flavor industry, whereas their ability to produce AHL-degrading bacterial enzymes, which is known *in vitro*, remains to be evaluated *in situ* so that they can be used as QS inhibitors (5, 14, 50, 56, 82, 83, 99, 100, 101, 116, 117, 124). Inhibitory substances or compounds that may mask the QS effect have been reported in foods of animal origin (5, 116, 117). In general, each food product or class of products will suit a type or group of QSIs that might be used as alternative preservatives to prevent or delay food spoilage. One dynamic direction of research is to model the cell-to-cell communication in a food matrix, adding QSIs (commercially available or isolated in the laboratory) directly to the food matrix and also taking into account the spatiotemporal behavior and type of growth of these cells, as well as all biotic and abiotic factors to predict the shelf life of foodstuffs. In these ecosystems, factors such as habitat, niche domain, microbial interactions, and community behavior should be included in studies relevant to the role of QS.

RESEARCH NEEDS AND PERSPECTIVES

In this minireview, a summary of the results of different studies related to the contribution of QS in the behavior of microbes in the food chain is provided. A significant issue encountered in this context is the lack of common research targets, e.g., which pathogenic and/or spoilage bacteria will be studied and why. For instance, *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* could be the target organisms as far as pathogenic bacteria are concerned, while *Pseudomonas* spp. and *Enterobacteriaceae* can be used to study spoilage bacteria. The annual health care costs caused by a selected few food-borne pathogens, such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* sp., has been estimated at 5 to 6 billion euros per year, of which 4 billion are attributed to meat and meat products (28, 108). Similar reports are related to spoilage by pseudomonads and enterobacteria (58). For the above-mentioned reasons, a selection of organisms for collaborative and/or comparative studies should be defined. Another issue should be the standardization of methodologies and tools that will be used to assess and compare the biological effects of such signaling compounds. However, the main difficulties and limitations in setting up experiments to expand knowledge of the QS effect in the food sector beyond the state of art are related to the following: (i) the spatial heterogeneity of the food matrix, (ii) the so-called "quantal or quantum microbiology," i.e., the "bridge" between the uncertainty and heterogeneity of individual cells (individuality) in microscale and the superficial stability and homogeneity of large populations in macroscale (77), and (iii) the limitations in the qualitative and quantitative estimation of QS compounds. Some alternative approaches that could be used to address the above issues are detailed below.

(i) Spatial heterogeneity of the food matrix. In most studies, the spatial heterogeneity of the food matrix has not been taken into account, and consequently, it is thought that cells are exposed to the same concentration of signal molecule. This cannot be the case with a food system, where, as mentioned previously, cells exist as planktonic, sessile, immobilized, or even constrained forms (137), and most of the sensing takes place in highly diverse communities that are living in a dynamic, i.e., spatially heterogeneous environment that changes in space and time. Clusters of cells are influenced by the complex spatial structure of diffusion spaces that change over time and affect the temporally changing spatial distribution of cells that produce a given autoinducer at a basal or induced rate (43). Thus, it could be possible that a nonessential parameter for life in liquid (planktonic), such as nutrient diffusion could be substantially important for immobilized cells. Thus, in food ecosystems, underestimated or overestimated parameters should be investigated in detail for every microsystem in order to gain a better understanding of and obtain insight into the exact role of QS in every case. For example, the identification of the proper variables (e.g., porosity, viscoelastic properties, etc.) characterizing the effect of structure (matrix) and physicochemical attributes of foods (e.g., pH, water activity, ability of nutrients and/or metabolites to diffuse) on microbial behavior, the spatial and temporal heterogeneity of bacteria, the variability in the physiological stage in the strains, and the succession of the microbial community in time, as affected from the implicit factors should be carefully investigated. This can be achieved if suitable methodologies for studying the effects of QS signaling compounds on food spoilage are developed and standardized. Such methodologies should offer adequate resolution in monitoring the behavior of microbes and in the identification of the release (producers) and sensing (reporters) of QS compounds. So far, two basic approaches have been used. The first approach uses mutants deficient in QS signaling and strains producing QS compounds to

help identify the effect of each type of signaling molecule on growth kinetics and more specifically on the growth determinants of the various organisms tested (i.e., target genes and phenotypes). The second approach is indirect monitoring of changes in microbial activity or growth, e.g., fast growth episodes accompanied by a high reproductive effort (*r* strategy or high maximum growth rate [μ_{max}]) or carrying capacity (*k* strategy) (i.e., slow growth and low productive effort) (8, 12). The latter approach, which can be applied in liquid food systems (e.g., milk), could potentially lead to the use of these autoinducers as novel antimicrobial agents and compounds to control microbial growth, survival, and virulence in such systems. This is due to the fact that by this indirect method, factors such as impedance (15, 26, 86, 132), the composition of the medium, the history of the cells, and the variability of the population can be used to evaluate the effects of QS signaling compounds, either synthetic or naturally produced, from different food systems, on the growth kinetics (for instance, the *k* or *r* strategies) of various spoilage and pathogenic bacteria. On the other hand, in the case of solid foods, systems that mimic a food matrix (e.g., gel cassette [111]) can be useful tools and model food setups in the evaluation of questions such as (i) how in these dynamic systems, numerous different QS systems that coexist act synergistically or interfere with each other, (ii) whether the various food components promote or inhibit some QS systems and enhance others, and (iii) whether the food microarchitecture dramatically influences cell-to-cell communication and consequently the spoilage mechanism (for example, are the molecules [e.g., QS compounds] produced at each stage of microbial development a consequence of the strategy that a single cell can follow to recognize the environment beyond itself the major determinant of species succession in the microbial community or vice versa?).

(ii) Probalistic microbiology or "quantal microbiology." Most studies have been designed to use large inocula (populations), and although the composition of the growth medium may vary, in most cases it is considered *a priori* that the physiological status of cells is similar, that all cells produce signal molecules at the same rate, or that they are all exposed equally to these compounds. However, individual-based modeling using microscopic techniques (e.g., direct imaging of cells) or 2-fold dilution protocols to obtain single cells that may be added in a liquid culture of Bioscreen or on the surface of agar on a gel cassette (61, 62, 75, 109) may elucidate the true heterogeneity of a (theoretical) homogeneous population and prove that the effects of QS compounds on single cells are also stochastic, rather than deterministic, as is the macroscopic behavior of bacteria. This way, the extreme individual responses of single cells behaving as "outliers" of a larger homogeneous population and masked by adjacent cells showing an "average" behavior may be revealed when cells are studied individually.

In particular, when experiments are carried out with millions of bacteria and virus particles, it is possible to learn a great deal about the interaction of the pair by taking averages; however, the action of a single cell of *Listeria monocytogenes* for example cannot be predicted. The power of single-cell studies was illustrated dramatically by Stephens et al. (119), who used an automated growth analyzer to measure the recovery times of heat-injured salmonellae and showed that with single-cell inocula, the lag phase can vary widely in the length of time, even using identical media. When the inoculum was increased 1,000-fold, the lag phase shortened dramatically. Replicate results with low inocula were consistent for single broth preparations but not with different batches of the same broth from the same manufacturer. The technique proved to be an exquisitely fine tool to demonstrate cell-to-cell variability and minute differences in available nutrients or other conditions for stressed cell recovery. In fact, the inoculum effect (IE) *per se* is a very important issue, at least among food microbiologists dealing with quantification of kinetic parameters, such as lag and μ_{max}, of spoilage and pathogenic bacteria in food systems. So far, the IE and the degree of heterogeneity and/or diversity in the population are ignored because the researcher is measuring the average response of the population, i.e., in a deterministic way. This could be problematic, as it has been well established that not all signaling compounds display similar activities in different strains; variation in membrane composition, secondary regulation of gene expression, and the presence of competing ligands may have a large impact on the observed biological effects of a QS compound, and such effects are masked in larger populations, because the response of a large population commonly represents the behavior of the "best performer" if the rate of growth or the growth limit is the dependent variable or of the worst-case scenario if resistance to stress is examined. Therefore, a direct comparison of activities of QS compounds obtained from different studies can be misleading and is not appropriate in many cases.

(iii) Assays used. Another point to note is that bacterial species utilizing the same general type of quorum sensor (the same general signals and receptors [for example, AHL-based signaling]) should not be necessarily expected to respond in similar ways when exposed to a given chemical probe. That is, any structure-activity trend may be species dependent rather than system dependent. Such information is valuable for the identification of both selective and broad-spectrum, multispecies modulators of quorum-sensing activity (33, 102).

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