Quorum Sensing in the context of food microbiology

Panagiotis SKANDAMIS, and George-John E. NYCHAS*

Department of Food Science & Technology, Agricultural University of Athens, 75 Iera Odos, 11855, Athens, Greece.

Key words: Quorum sensing; autoinducer; N-acyl homoserine lactone; furanosyl borate diester; autoinducing peptide; bioassay; food spoilage; quorum quenching; food ecology.

* Author for correspondence

Running title: Role of quorum sensing in food spoilage
Table of Contents

21 Abstract 3
22 BACTERIAL COMMUNICATION 4
23 GROUP OF COMMUNICATION COMPOUNDS 6
24 INTRASPECIES CELL-TO-CELL COMMUNICATION 7
25 INTERSPECIES CELL-TO-CELL COMMUNICATION 8
26 QUORUM SENSING IN THE CONTEXT OF FOOD AND FOOD PROCESSING 9
27 Microbial ecology of food contact surfaces: 9
28 Food Microbial ecology: 11
29 Microbial spoilage in foods of animal and plant origin; 15
30 PROMOTING vs QUENCHING Quorum Sensing 15
31 RESEARCH NEEDS - PERSPECTIVES 17
32
33
34
35
36
Abstract

Food spoilage may be defined as a process, which renders a product undesirable or unacceptable for consumption, is the outcome of the biochemical activity of 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 of these communication systems, thereby reducing or preventing e.g., spoilage reactions or even controlling the expression of virulence factors. Due to the abundant literature reports on the QS fundamental features, e.g. chemistry and definitions of QS compounds, in this review, we only allude to the types and chemistry of QS signalling molecules per se as well as 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 co-ordination of spoilage activities and thus may be used as biopreservatives and (iv) the future experimental approaches that need to be undertaken in order to explore the “grey” or “black” areas of QS, increase our understanding of how QS affects microbial behaviour 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 considered as populations of cells that act individually, but it is now increasingly apparent that there is much interaction and communication among adjacent cells (55). Quorum Sensing (QS), a term introduced by Fuqua and Winans (32) to describe cell-to-cell communication, is the mechanisms used by bacteria to understand changes in their environment and consequently to apply specific strategies that allow adaptation to environmental stress in space and in time. This continuous adaptation process may be affected by microbial communication (136). 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 behaviours 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, e.g. 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 due to 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 intra-species 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 as 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 regards to pathogenic Gram-negative bacteria, including the genera *Agrobacterium*, *Brucella*, *Bukholderia*, *Erwinia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia*, *Vibrio*, and *Yersinia*, the QS mechanism for the regulation of virulence factors synthetis has been exploited (132). This mechanism has also been used by *Bacillus*, *Enterococcus*, *Staphylococcus*, *Streptococcus*, *Streptomyces*, and *Rhizobium* genera, to develop genetic competence, produce antimicrobial peptides or exotoxins, for biofilm formation, and nitrogen fixation (45, 95). Bacteria not only communicate with members of the same species, but may also “eavesdrop” the “conversation” of other species and modulate their behaviour in response to signal molecules they do not synthesize (29).

As it was 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, etc. Conversely, much less attention has been paid to the ecological context of why bacteria produce signalling molecules and respond to both intra- and interspecies signals, and even less studies have 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 signals 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. (115),
for example, reported that the survival and virulence expression of a luxS mutant strain of *E. coli* O157:H7 was enhanced in the presence of AI-2. Similarly, production of AI-2 by *S. enterica* serovar Typhimurium contributed significantly to its ability to colonize in chicken intestine compared to the LuxS’ mutant strain (9).

Disrupting the QS path 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 (QSI) 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 signalling 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 behaviours 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 (AI-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, 138); (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 (75,120).

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 cells of multi-celled organisms, suggesting their role in bacterial conversation with plant and animal cells rather than with other bacteria. 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 AHL and this may be one of the reasons why relatively few synthetic AHL-based derivatives capable of exhibiting heightened activities relative to native AHLLs have been identified. This is a particular concern for exploited 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 C1 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 C3 position (133). AHLs are synthesized with the reaction of S-adenosyl-methionine (SAM; essential metabolite in the central metabolism) with an acy-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 down-regulation of multiple target genes (123). Bacterial species may synthesise 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. length and substitution or not of the C3 of the acyl chain) of AHLs, including temperature, pH, NaCl, growth media, inoculum size and bacterial growth phase (37, 76, 77, 79, 142).

In Gram-positive bacteria, cell-to-cell communication is accomplished via peptides or modified peptides (auto-inducing peptides - AIPs). 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 (AI-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 cyclises 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 e.g. more resistant to antimicrobial agents, to cleaning agents and other antimicrobial substances, over 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 transcriptional programs from free-living planktonic bacteria of the same strain (3, 34).

A growing body of evidence demonstrates that QS contributes to biofilm formation by many different species (21, 36, 40, 73, 96). 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 between 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, contrary to
the laboratory studied pure-species biofilms (10, 97, 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
hall 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 (36). The EPS production is required for cell aggregation that leads to biofilm formation,
respectively. This was not confirmed in Gram-negative bacteria isolated from food processing
environments (127). Though signaling molecules have been detected in biofilms, yet their precise
role in biofilm formation is still not clear. Further studies under controlled in vitro conditions,
involving the effect of specific QS signals in mono- 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 environment 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 foodborne pathogens on food contact surfaces could open new research
avenues towards 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 the first 24 h, compared to 0% or 20% CFS. Thin-layer chromatography revealed the existence of signalling compounds, in the form of acylhomoserine lactones (AHLs), in the two growth media containing CFS (20 and 50%), during whole 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:**

Food matrix should be considered among those environments, where quorum or other sensing molecules are released have not 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, 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, 67, 70, 93, 123). Although the production of these compounds has been attributed to certain members of the food microbial association e.g., Pseudomonads, Enterobacteriaceae, and lactic acid bacteria, 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 (SSO) or ephemeral spoilage organisms (ESO) are terms that have been used to describe the fact that only a small fraction of microorganisms that temporally dominate or their succession in a food ecosystem at the time of spoilage (8,85).

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 or attachment, or a combination thereof. As a result, the cells are immobilised and localised 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 result in a series of interconnected micro-environments, some of which may be preferential for microbial growth (138). With the possible exception of highly processed products, foods harbour 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 (7) and the in situ cell-to-cell ecological interactions, which often occur in the solid phase of foods.

Thus, the food microbial ecology approach is pertinent to the analysis of cell-to-cell communication in different food ecosystems, for example: (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? (ii) 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 co-exist 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 similar way even if in some cases the SSOs or ESOs are the same (77, 79). Since the confirmation of presence/absence or the determination of levels of QS compounds, even when advanced analysis is carried out, e.g., GC-MS, HPLC-MS 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 the effects of autoinducer compounds should be applied. These questions should be addressed to 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, as compared to diffusion of metabolites away of cells in a liquid culture (57, 76, 125). Thus, considering that growth within a colony results in inevitable proximity of cells and increase in cell density at a limited space, the release of QS-compounds and their rapid diffusion within the colony might be more directly sensible and thus, have greater impact on immobilized 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, affected by the presence of acylated homoserine lactones (AHLs) and autoinducer-2 (AI-2) signalling compounds and/or other novel signals existing in Cell Free Supernatant (CFS), produced by pathogenic and spoilage bacteria e.g. Pseudomonas aeruginosa, Yersinia enterocolitica-like GTE 112, Serratia proteamaculans 00612, Y. enterocolitica CITY650 and Y. enterocolitica CITY844. It was shown that (i) the growth
kinetic parameters as well as the microbial activity of four Salmonella strains were affected by the addition of CFS produced by other pathogenic and spoilage bacteria; and (ii) there was not a uniform type of response in the bacterial strains tested meaning that the effect of AHLs or AI-2 signalling 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 e.g. provide 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, 97) 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 signalling 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 under-production of substances necessary for growth (e.g. enzymes, metabolites etc) (104). It needs to be noted however that other unknown non-signalling 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 real situations of interactions between bacteria and signalling 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 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 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 the rate of spoilage in these products (84, 85, 87). This due to the fact that only the depletion of these compounds affects the activity of extracellular proteolytic enzymes and thus, may influence both the development of microbial community and the habitat and activity domain (i.e., microbial “domain”) (7, 67). On the other hand, the spoilage of vegetables and fruits, which is commonly manifested as visual defects including enzymatic browning, off-flavour/off-odours and/or texture breakdown is often caused by the pectinolytic activity of *Pseudomonadaceae* or *Enterobacteriaceae* (mostly *Erwinia* spp.) growing to high cell densities (10^8–10^9 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, due to spoilage being a phenomenon requiring high levels of microbial populations, QS may be a potential regulatory spoilage mechanism. For this reasons, the potential role of QS in spoilage has been investigated, although by a limited number of studies as indicated in Table 1.

**PROMOTING vs QUENCHING Quorum Sensing**

Food spoilage is considered to be a process, which renders a product undesirable or unacceptable for consumption. This complex ecological phenomenon is the outcome of the biochemical activity,
through various enzymes of microbial association, which will eventually dominate according to the prevailing ecological determinants on each food system (Nychas et al. 2008). 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 (30, 53, 94, 101, 105, 126, 128, 129), 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 they are already too many and hence should cease their growth and/or metabolic activity. Such approaches 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), 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 these reported from 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 meat storage as well as on (ii) 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 QSI e.g., halogenated natural furanones or synthetized derivatives, have been extensively investigated and have been successful applied for preventing toxin production, minimizing bacterial resistance, inhibiting expression of virulence factors etc. (73), 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 (43) 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, 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 identified as acyclic disulphur 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 produce AHL-degrading bacterial enzymes which is known in vitro, it remains to be evaluated in situ so as to 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 QSI 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 directly QSI (commercially available or isolated in the laboratory) 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 behaviour should be included in studies relevant to the role of QS.

**RESEARCH NEEDS - PERSPECTIVES**

In this review, a summary of the results of different studies related to contribution of QS in the microbial behavior 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* can be the target organisms as far as the pathogens is concerned while Pseudomonas spp., and Enterobacteriaceae
can be used to study spoilage. The annual health care costs, traced to a few selected food-borne pathogens such as *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* sp., has been estimated at 5-6 billion € per year, of which 4 billion are attributed only to meat and meat products (28, 106). 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 as consequence to compare the biological effects of such signaling compounds. However, the main difficulties and limitations in setting experimental plans to expand the knowledge on the QS effect in the food sector beyond the state of art, are related to: (a) the spatial heterogeneity of food matrix, (b) the so-called “quantal or quantum microbiology” i.e., the “bridge” between the uncertainty and heterogeneity of individual cells (individuality) in micro-scale and the superficial stability and homogeneity of large populations in macro-scale (77) (c) 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 food matrix;** In most studies this have not taken into account and as consequence it is considered 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 (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. Cells clusters are influenced from the temporally changing, complex spatial structure of diffusion spaces and do affect the temporally changing spatial distribution of themselves that produce a given autoinducer at a basal or induced rate (43). Thus it could be possible that a non-essential parameter for liquid type of life (planktonic) e.g. nutrient diffusion could be proved substantially important i.e., for immobilized cells. Thus, in food ecosystem,
underestimated or overestimated parameters should be investigated in details, for every different micro-system in order to receive answers and obtain insights of 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 be diffused) on microbial behavior, the spatial and temporal heterogeneity of bacteria, the variability in the physiological stage among the strains as well as 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 effect of QS signaling compounds on food spoilage should be developed and standardized. Such methodologies should offer adequate resolution in monitoring the microbial behaviour and in the identification of the release (producers) and sensing (reporters) of QS-compounds. So far two basic approaches have been followed (a) the use of mutant, deficient in QS signaling, in parallel with the QS producing strains could contribute identifying the effect of each type of signaling molecules on growth kinetics and more specifically on the growth determinants of various tested organisms (i.e. target genes and phenotypes) (b) the indirect monitoring of the changes in microbial activities growth, e.g. fast growth episodes accompanied by a high reproductive effort ($r$-strategy or high $\mu_{max}$) or carrying capacity ($k$-strategy) (i.e. slow growth and low productive effort) (7, 12). The latter approach, which can be applied in liquid food system e.g. milk, could potentially lead to the exploitation 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 in this indirect method e.g. impedance (15, 26, 86, 132), the composition of the media, the history of the cells, the variability of the population can be used for the evaluation of the effect of QS signaling compounds, either synthetic or naturally produced, from different food system, on the growth kinetics e.g. $k$ or $r$ strategies, of various spoilage and pathogenic bacteria. On the other hand, in the case of solid foods, systems that mimic food matrix e.g. gel cassette (134), can be proved useful tools and model food
set-ups in the evaluation of questions such as (i) how in this dynamic systems, numerous different
QS systems that are coexisting acting 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 e.g. are the molecules (e.g., QS compounds) produced at each stage of
microbial development, as a consequence of the strategy that a single cell can follow to recognize
the environment beyond its cell, 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 inoculum (populations) and although the composition of the growth
media 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 (60, 61, 75,109) may
elucidate the true heterogeneity of a (theoretical) homogeneous population and prove that the effect
of QS-compounds on single cells is 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 studying cells 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 for example of Listeria monocytogenes cannot be predicted. The power of single-cell
studies was illustrated dramatically by Stephens et al. (119), using an automated growth analyzer to
measure the recovery times of heat-injured salmonellae where it was shown that with single cell
inocula, the lag phase can vary widely in length of time, even using identical media. When the
inoculum was increased 1000-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 it is very important issue, at least
among food microbiologists dealing with quantification of kinetic parameters e.g. lag, $\mu_{\text{max}}$, of
spoilage and pathogenic bacteria on food systems. So far, this IE as well as the degree of
heterogeneity and/or diversity in the population is ignored because the researcher is measuring an
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 compounds
and such effects are masked in higher populations, because the response of large population
commonly represents the behavior of the “best performer” if the rate of growth or the growth limits
are the dependent variables, or of the worst case scenario if the 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: An additional point to note is that bacterial species utilizing the same general type
of quorum sensor (that is, 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 trends 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, 101).
References


28. EFSA 2009


108. Scallan et al. 2011


142. Zhao, L., Montville, T.J. and D.W. Schaffner 2006. Evidence for quorum sensing in Clostridium botulinum 56A. Letters in Applied Microbiology 42:54-58,
<table>
<thead>
<tr>
<th>QS studies on</th>
<th>QS signal response based on the bioassay performed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meats and meat products</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em> strains isolated from vacuum-packed chilled meat</td>
<td>C6-3-oxo-HSL, C6-HSL</td>
<td>Gram et al., (38,39)</td>
</tr>
<tr>
<td>Meat extracts and isolated <em>Enterobacteriaceae</em> strains from chill-stored vacuum-packed meat</td>
<td>C6-3-oxo-HSL, C6-HSL</td>
<td>Bruhn et al., (11)</td>
</tr>
<tr>
<td>Food samples e.g. beef, chicken, turkey products (AI-2-like activity)</td>
<td>Borated AI-2</td>
<td>Lu et al., (70)</td>
</tr>
<tr>
<td>Pseudomonad and <em>Enterobacteriaceae</em> isolates from aerobically chilled-stored proteinaceous raw foods</td>
<td>Medium- and long-side chain AHLs</td>
<td>Liu et al., (67)</td>
</tr>
<tr>
<td>AHL signals during storage of aerobically chill-stored ground beef</td>
<td>C4-HSL, C6-3-oxo-HSL and undefined AHLs</td>
<td>Liu et al., (67)</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em> strains isolated from meat</td>
<td>C4-HSL</td>
<td>Medina-Martinez et al., (77)</td>
</tr>
<tr>
<td>AHL production of <em>Yersinia enterocolitica</em> strains in fresh beef and pork extracts</td>
<td>C6-3-oxo-HSL, C6-HSL</td>
<td>Medina-Martinez et al., (77)</td>
</tr>
<tr>
<td>Poultry meat-derived fatty acids, as inhibitors to AI-2</td>
<td>Borated AI-2</td>
<td>Widmer et al., (135)</td>
</tr>
<tr>
<td>Survival and virulence gene expression of <em>E. coli</em> O157:H7 in the presence of AI-2 and ground beef extracts</td>
<td>Borated AI-2</td>
<td>Soni et al., (116,117)</td>
</tr>
<tr>
<td>Ground beef–derived fatty acids, as inhibitors to AI-2</td>
<td>Borated AI-2</td>
<td>Soni et al., (116,117)</td>
</tr>
<tr>
<td>Cell-free extracts from minced pork stored aerobically at 5 and 20 °C</td>
<td>Short-, medium- and long-side chain AHLs, AI-2</td>
<td>Nychas et al., (86)</td>
</tr>
<tr>
<td><em>Pseudomonas fragi</em> isolated from fresh and spoiled meat</td>
<td>Borated AI-2</td>
<td>Ferrocino et al., (30)</td>
</tr>
<tr>
<td>Production of quorum sensing signals by <em>E. coli</em> O157:H7 strain in meat broths (beef and pork)</td>
<td>AI-2 activity</td>
<td>Silagyi et al., (110)</td>
</tr>
<tr>
<td>Lactic acid bacteria isolated from minced beef packaged under modified atmospheres</td>
<td>AI-2-like activity</td>
<td>Blana et al., (5,6)</td>
</tr>
<tr>
<td><strong>Vegetables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detection of AHL in bean sprouts stored at 5°C</td>
<td>Broad range of AHLs</td>
<td>Gram et al., (39)</td>
</tr>
<tr>
<td>AI-2-like activity in fresh foods (tomato and carrot)</td>
<td>AI-2 activity</td>
<td>Lu et al., (70)</td>
</tr>
<tr>
<td>Extracts from commercial bean sprouts</td>
<td>C6-3-oxo-HSL</td>
<td>Rasch et al., (99,100)</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em>, pseudomonads and <em>Vibrionaceae</em> strains isolated from commercial bean sprouts</td>
<td>C6-3-oxo-HSL, C10-3-oxo-HSL, C10-3-hydroxy-HSL, C4-HSL, C6-HSL, C8-HSL, C10-HSL and undefined AHLs</td>
<td>Rasch et al., (99,100)</td>
</tr>
<tr>
<td>Presence of AI-2-like activity in cell free supernatants of eggplant, squash, tomato, pepper, cucumber, potato and carrot</td>
<td>AI-2-like activity</td>
<td>Lu et al., (71)</td>
</tr>
<tr>
<td>AHL production of <em>Yersinia enterocolitica</em> strains in lettuce and cucumber extracts</td>
<td>C6-3-oxo-HSL, C6-HSL</td>
<td>Medina-Martinez et al., (77,79)</td>
</tr>
<tr>
<td>AHL production of <em>Aeromonas</em> spp. in simulate agar of broccoli, parsley and spinach</td>
<td>C4-HSL</td>
<td>Medina-Martinez et al., (77,79)</td>
</tr>
<tr>
<td>Production of quorum sensing signals by <em>E. coli</em> O157:H7 strain in spinach broth</td>
<td>AI-2 activity</td>
<td>Silagyi et al., (110)</td>
</tr>
</tbody>
</table>