Review paper

Mechanism and manifestation of bacterial quorum sensing in food environment

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Abstract

Research in the field of food science and technology has shown that bacteria can communicate with each other. This communication is population-density dependent and involves signalling molecules that can diffuse between the bacterial cells. The process of communication is called 'quorum sensing'. Quorum sensing is now known to be a widespread phenomenon responsible for modulating many different activities in diverse Gram-negative and Gram-positive bacteria. This phenomenon has been found to be responsible in food spoilage and biofilm formation by food-related bacteria in the food industry in addition to pathogenesis. Understanding bacterial quorum-sensing systems can help in controlling the growth of undesirable food-related bacteria. Blocking the quorum-sensing signalling molecules in food related bacteria may possibly prevent quorum-sensing-regulated phenotypes responsible for food spoilage. With proper understanding of quorum sensing mechanism we can identify quorum-sensing inhibitors that could be used as food preservatives to enhance food safety and increase shelf life.

Keywords: Communicate, signal, phenotype

Introduction

Bacteria carry with them a myth that they are an unambitious unicellular entity whose single motto in life is to grow and divide *ad infinitum* in isolation. The evolved traits of collective social behavior and communication were believed to be only for multicellulars. This myth has been shattered with path breaking research confirming that bacterial species do coordinate communal behavior. It all began with a brilliant observation of growth kinetics of marine bioluminescent bacteria that the onset of exponential growth occurs without a lag but bioluminescence does not increase until mid-logarithmic phase, probably due to an inhibitor in the medium (Farghaly, 1950). Another study concluded that the inhibitor attached to the luciferase and the process is regulated by extracellular secreted signal components (Kempner and Hanson, 1968). The signal molecule was dubbed as 'autoinducer' and the desired response as 'autoinduction'. In 1970, Nealson and colleagues reported that luminescence in the marine symbiont bacterium *Vibrio fischeri* that inhabit light organs of animals such as *Euprymna scolopes,* was produced only at high cell density. They were the first to propose the phenomenon of cell density-dependent autoinduction. The term "quorum sensing" specifically refers to the cell-density linked, coordinated gene expression in populations that experience threshold signal concentrations to induce a synchronized population response (Fuqua *et al*., 1994).

There are many situations where the ability of a bacterial population to behave co-operatively could be highly

advantageous particularly in the contexts of conjugation, symbiosis, niche adaptation, production of secondary metabolites, combating the defense mechanisms of higher organisms and for facilitating population migration where the prevailing conditions have become unfavorable.

Bacterial language is chemical in nature using certain signaling molecules. They possess specific receptors which can detect these autoinducers. Upon binding of inducer to receptor, it activates transcription of certain genes, including those for inducer synthesis. When only a few bacteria are present, diffusion reduces the concentration of the inducer in the surrounding medium to almost zero. However, as the population grows the concentration of the inducer passes a threshold (quorate) and the receptor becomes fully activated. Activation of the receptor induces the up regulation of specific genes, causing all of the cells to begin transcription at approximately the same time.In this review, all these aspects have been described in context of food.

Decoding the language

Signal molecules implicated in cell-to-cell communication are now known as autoinducers or quorum-sensing molecules, and their function is to regulate gene expression in other cells of the community, which, in turn, control a number of bacterial responses. Molecules implicated in quorum sensing (QS) possess the ability to induce their own production ,hence are known as 'autoinducers' (AI) some of the moleculars used by bacteria for quorum sensing are listed in Table 1. Cell-to-cell signaling systems are broadly grouped into 4 main categories. Two of these systems, which use autoinducer-1 (AI-1) and autoinducer-3 (AI-3), are found in Gram-negative cells, while the Gram-positive bacteria use a 3rd type of signaling system,

the autoinducing polypeptide (AIP) system. The 4th system, using autoinducer-2 (AI-2), is found in Gram positive as well as Gram-negative bacteria. It can be said that AI-2 is used for interspecies communication.

The systems have a common flow of events (Sifri, 2008)

- Production of small biochemical signal molecules by the bacterial cell;

- Release of the signal molecules, either actively or passively, into the surrounding environment;

- Recognition of the signal molecules by specific receptors once they exceed a threshold concentration

- Changes in gene regulation

- The autoinducer molecules important in quorum sensingare are summerized here.

Autoinducer 1: Acyl homoserine lactones are the major group of autoinducer signals in gram-negative bacteria. They have a conserved homoserine lactone (HSL) ring with a variable acyl side chain. The length and saturation level of the acyl chains coupled to the presence or absence of oxo or hydroxyl substitutions at the C-3 position of the acyl chain provide variation and specificity for quorum-sensing communication in a mixed bacterial population (Shaw *et al*., 1997)

Autoinducer 2 has been discovered in many gram-negative bacteria as a global signal molecule for interspecies communication, as it is made by gram-positive as well as gram-negative bacteria (Bassler, 1999). AI-2 is identified it as a furanosyl borate diester. The proposed structure contains two fused five member rings containing one boron atom bridging the diester (Chen *et al.*, 2002).

Table1: List of signal molecules used by bacteria for quorum sensing

Organism	Signal molecule	Function
Vibrio fischeri	3-Oxo-C_6-HSL	Bioluminescence
Aeromonas hyrophila	N-butanoyl-HSL	Serine protease and metalloprotease production
Aeromonas salmonicida	N-butanoyl-HSL	exoprotease
Agrobacterium tumefaciens	3-Oxo-C_6-HSL	Ti plasmid conjugal transfer
Burkholderia cepacia	N-octanoyl-HSL	Protease and siderophore porduction
Chromobacterium violaceum	N-hexonoyl-HSL	Hydrogen cyanide, antibiotics,
Erwinia caratovora	3-Oxo-C_6-HSL	Pectinase synthesis
Pseudomonas aerofaciens	N-hexonoyl-HSL	Phenazine antibiotic biosynthesis
Pseudomoans aeroginosa	3-Oxo-C_6-HSL	Virulence, biofilm formation.
Rhizobium leguminosarum	N-hexonoyl-HSL	Rhizosphere genes
Serratia liquefaciens	N-butanoyl-HSL	Swarmer cell differentiation
Yersinia pseudotuberculosis	N-octanoyl-HSL	Bacterial aggregation and motility
Rhodobacter sphaeroides	7,8-cis-N-(Tetradecanoyl)-HSL	Prevents bacterial aggregation

Sources: Raina *et al.*, 2009, Hammer and Bassler, 2003

Auotinducer 3: Epinephrine/norepinephrine/AI-3 signaling is used as an interkingdom chemical signaling system between microbes and their hosts. This system is also exploited by pathogens to regulate virulence traits. In enterohemorrhagic *E. coli* (EHEC) O157:H7, it is essential for pathogenesis and flagella motility. These three signals activate expression of a pathogenicity island named Locus of Enterocyte Effacement (LEE), Shiga toxin, and the flagella regulon (Moreira and Sperandio, 2010)

Cyclic dipeptides. Identified in strains of *Pseudomonas* these new signal molecules were the diketopiperazines (DKPs) cyclo (L-Ala-L-Val) and cyclo(L-Pro-L-Tyr).

Autoinducing Peptides: Gram-positive species predominantly utilize small post translationally modified peptides for cell-to-

cell signaling. These are exported via ATP-binding cassette (ABC)-type transporters The genes encoding the precursor peptide, membrane bound sensor kinase protein, and the transporter machinery are usually located in a single gene cluster (Bassler, 2002).

The LuxR/I signaling system:

The luciferase operon in *V. fischeri* is regulated by two proteins, LuxI, responsible for the production of the AHL (acyl-homoserine lactone) and LuxR a transcription factor responsible for controlling gene expression in the presence of the autoinducer. The schematic view of the mode of action of the system is shown in Figure 1. AHLs have a conserved homoserine lactone ring connected through an amide bond to
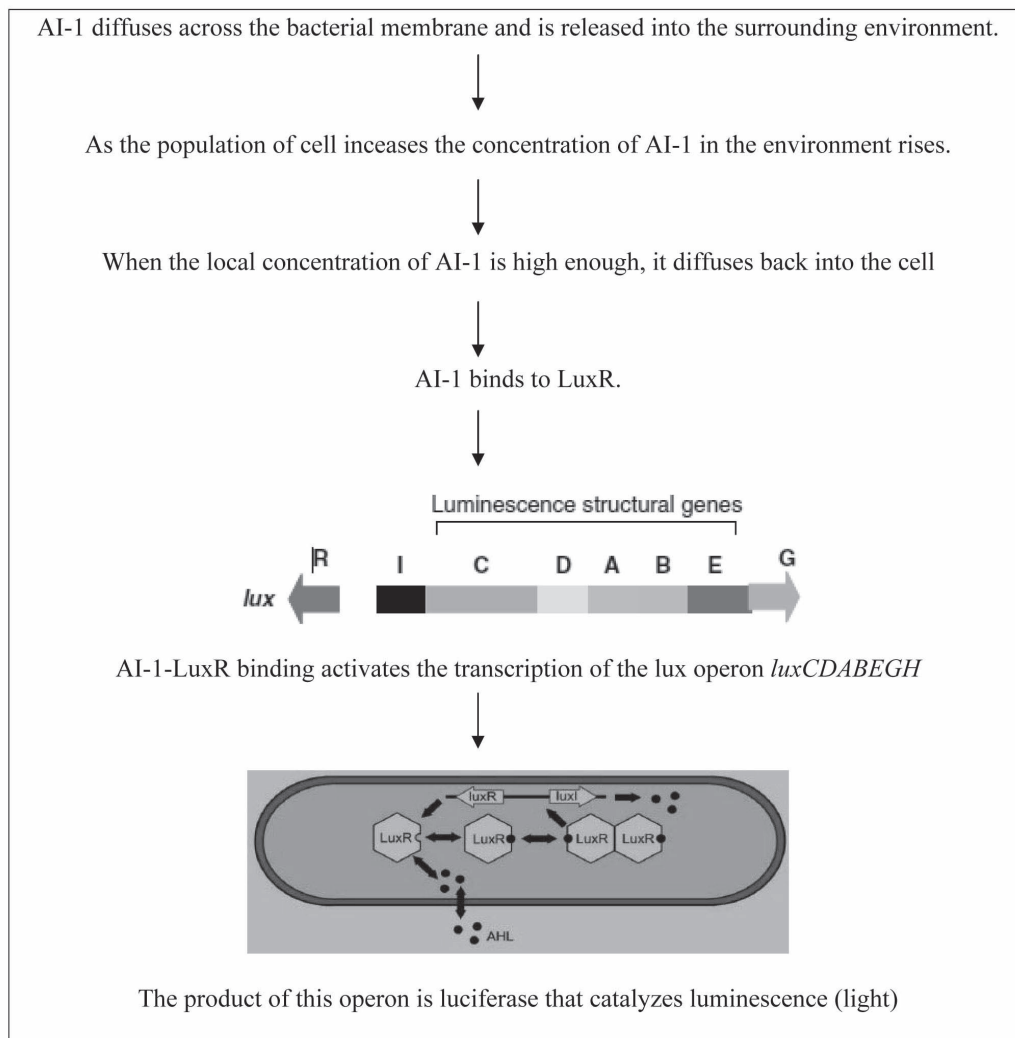
AI-1 diffuses across the bacterial membrane and is released into the surrounding environment.

As the population of cell inceases the concentration of AI-1 in the environment rises.

When the local concentration of AI-1 is high enough, it diffuses back into the cell

AI-1 binds to LuxR.

AI-1-LuxR binding activates the transcription of the lux operon *luxCDABEGH*

The product of this operon is luciferase that catalyzes luminescence (light)

Figure 1: The process of quorum induced bioluminescence by the LuxR/I system

a variable acyl chain. Acyl chains vary in number of carbons from four to 18 and the third position may or may not be modified (carbonyl group, hydroxyl or fully reduced). Different acyl chains ensure that different AHLs will be recognized by different LuxR-type proteins. The LuxR/I system was the first one to be described in *V. fischeri* (Nealson *et al.*, 1970). *P. aeruginosa* produces two AHLs, N-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL) and N-butanoyl-L-homoserine lactone (C4- HSL) (Pearson *et al.*, 1995). These AHLs bind to and activate LasR and RlhR transcription factors, respectively (Parsek and Greenberg, 2000).

Quorum sensing in Gram-positive organisms

Quorum sensing in Gram-positive organisms relies on autoinduction by small peptides, which interact with two component systems ultimately regulating gene transcription. These small peptides are usually products of oligopeptides that are cleaved and/or further modified before being exported from the bacterium by transporters. At threshold concentrations, the peptides are recognized by sensor kinases that initiate phosphous transfer to a response regulator. The peptides involved in Gram-positive QS are often specific for their cognate receptors. The QS system that *S.aureus* utilizes is one of the most studied systems in Gram-positive organisms. Currently, two quorum-sensing mechanisms have been explained in *S. aureus.*

1. The first quorum-sensing system consists of a seven-amino-acid peptide autoinducer RAP (RNAIII activating peptide) and TRAP (target of RNAIII-activating protein).

2. Second quorum-sensing mechanism regulated by agr, (accessory gene regulator) locus, composed of two divergently transcribed units, namely RNAII and RNAIII whose transcription is under control of the P2 and P3 promoters respectively. The RNAII unit encloses four genes, namely agrB, agrD, agrC and agrA. The genes agrB and agrD are involved in the autoinducer production

The accessory gene regulator (Agr) system regulates toxin and protease secretion in staphylococci. At low cell density, the bacteria express proteins required for attachment and colonization, and as the cell density becomes higher, this expression profile switches to express proteins involved in toxin and protease secretion (Novick, 2003). *Streptococcus pneumoniae* was one of the original Gram positive systems characterized. The discovery of an ABC transporter that secreted the activator for competence, comA, revealed a class of ABC transporters that contained N-terminal

proteolytic domains (Zhou *et al.*, 1995). This led to the characterization of the activator as Competence Inducing Factor (CSP). (Havarstein *et al.*, 1996).

The LuxS/AI-2 signaling system

Vibrio harveyi QS system constitutes a mix between components of Gram-positive and Gram negative systems (Figure 2). *Vibrio harveyi* QS system constitutes a mix between components of Gram-positive and Gram negative systems (Figure 3). It has two QS systems:

1. Autoinducer (AI-1) , an AHL, and is primarily involved in intraspecies signaling

2. Autoinducer is a furanosyl borate diester involved in interspecies signaling

The structure of the 2 AI-2 signals are determined as

1. furanosyl borate diester (BAI-2) used by *V. harveyi* to control luminescence

2. furanone([2R,4SL]-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran [R-THMF]) used by *S. typhimurium* AI-2 released by the bacterium accumulates in the cell's environment

The only genes shown to be regulated by AI- 2 in other species encode for an ABC transporter in

Salmonella typhimurium has system named Lsr (LuxS-regulated), responsible for the AI-2 uptake (Taga *et al.*, 2001). This ABC transporter is also present in *E. coli* and shares homology with sugar transporters (Figure 3a)

The AI-3/epinephrine/norepinephrine signaling system

Besides being used in bacterial interspecies signaling, AI-3 has an intrinsic role in interkingdom communication. AI-3 cross signals with the eukaryotic hormones epinephrine/norepinephrine in an agonistic fashion (Sperandio *et al.*, 2003). It was first found in as a compound found in spent media that activated the expression of genes involved in attachment of Entero Haemorrhagic E. coli (EHEC). The presence of AI-3 is also linked to the formation of attaching and effacing lesions by EHEC, loci of enterocyte effacement (LEE) operons located within the EHEC chromosome (Sperandio *et.al* 2003).

Enterohemorrhagic E. coli colonizes the large intestine where it causes attaching and effacing (AE) lesions. The AE lesion is characterized by the destruction of the microvilli and the rearrangement of the cytoskeleton to form a pedestal-like structure, which cups the bacteria individually. Enterohemorrhagic E. coli senses AI-3 (produced by the normal
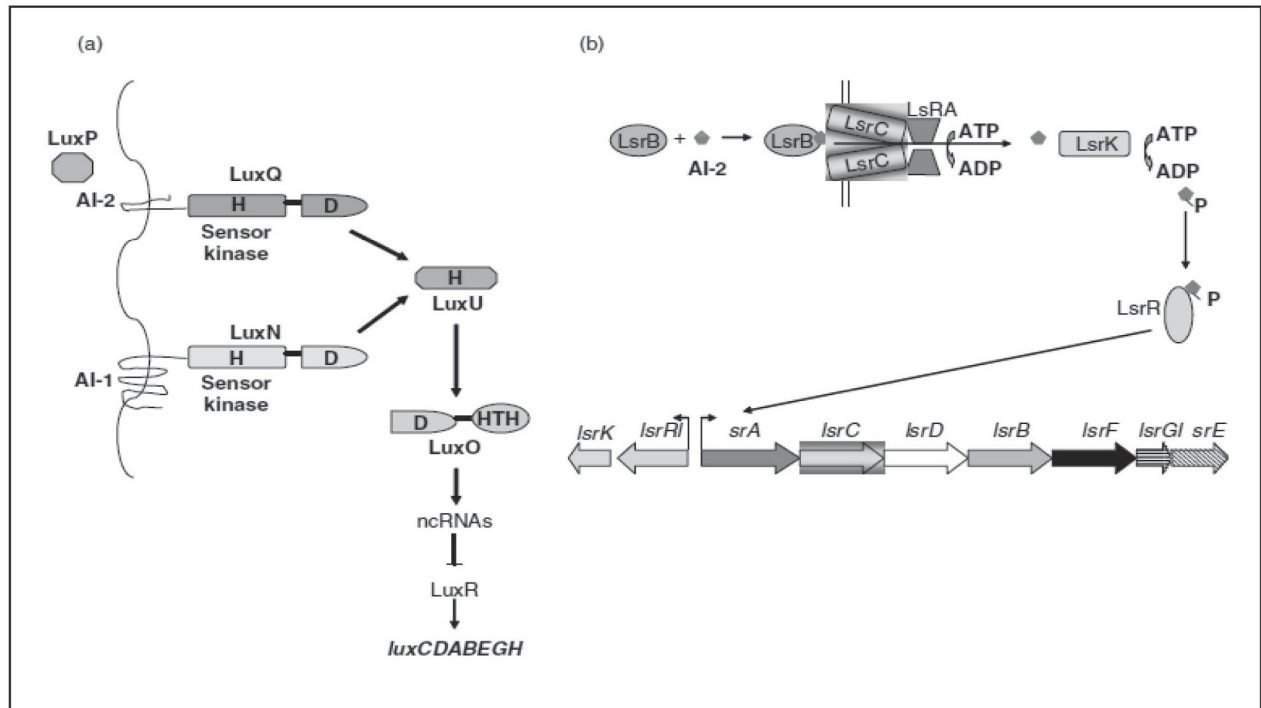
Figure 2: The LuxS/AI-2 quorum-sensing system. (a) *Vibrio harveyi* (b) in *Salmonella*, *E. coli*

V. harveyi has two hybrid sensor kinases, LuxN and LuxQ sensing AI-1 and AI-2, respectively.
LuxQ recognizes AI-2 complexed with its periplamic receptor LuxP.
↓
Upon sensing these signals, these kinases become phosphatases and the phosphorelay system (through LuxU and LuxO) is dephosphorylated.
↓
Consequently, LuxR mRNA is no longer degraded, and LuxR activates transcription of the luciferase operon.

Figure 3: Steps in the LuxS/AI-2 quorum-sensing system in *V.harveyi*

AI-2 receptor here is the LsrB (LuxS-regulated), periplasmic protein.
↓
Upon binding to LsrB, AI-2 is transported inside the cell
↓
AI-2 is then phosphorylated by LsrK, and presumed to interact with LsrR.
↓
LsrR is a transcriptional repressor of the lsr operon.
↓
Upon complexing of Lsr to AI-2, LsrR no longer represses lsr transcription

Figure 3a: Steps in the LuxS/AI-2 quorum-sensing system in *E.coli*

GI flora) and epinephrine/norepinephrine produced by the host to activate expression of the LEE genes and the flagella regulon (Sperandio *et al*., 2003). These signals are sensed by sensor kinases in the membrane of EHEC that relay this information through a complex regulatory cascade that activates the flagella regulon and the LEE pathogenicity island. The sensor for the flagella regulon is QseC that autophosphorylates in response to both epinephrine and

AI-3 and transfers its phosphate to the QseB response regulator, which in turn activates transcription of the flagella genes and itself (Clarke and Sperandio, 2005).

Repercussions of bacterial rendezvous

In food spoilage

In recent years, the detection of quorum sensing signals in

spoiled food products has added a new dimension to study the process of food spoilage. Several proteolytic, lipolytic, chitinolytic, and pectinolytic activities associated with the deterioration of foods are regulated by quorum sensing. Several types of signaling molecules have been detected in different spoiled food products. Hence, disrupting the quorum-sensing circuit can play a major role in controlling microbial gene expression related to human infection and food spoilage (Ragaert *et al*., 2007).Various signaling compounds, such as AI-1 and AI-2, have been detected in different food systems such as milk, meat, and vegetables (Bruhn *et.al* .,2004; Liu *et.al.,* 2006; Pinto *et.al.,* 2007).

Milk: In *Serratia proteamaculans* strainB5a, the production of extracellular lipolytic and proteolytic enzymes is under AHL-based quorum-sensing system, which implies the involvement of quorum sensing in the spoilage of milk by *Serratia* spp. (Christensen *et al*., 2003), Similarly, the production of AHLs in both raw and pasteurized milk by the psychrotrophic bacteria *Pseudomonas* spp., *Serratia* spp., *Enterobacter* spp., and *H. alvei* shows that quorum sensing may play a role in the spoilage of milk and dairy products (Whitfield *et al*., 2000). Moreover, the detection of furanosyl BAI-2 signals in significant amounts in regular milk containing a low bacterial population (10^2CFU/mL) suggests the possible involvement of interspecies communication in milk spoilage (Lu *et al*., 2004). Nisin production in *L. lactis* has been studied extensively, and it has been shown that it acts as an autoinducer. It regulates its own production at the transcriptional level with the involvement of a two-component regulatory system (for a review, see Kleerebezem, M. 2004). An example of interspecies communication via QS among LAB is represented by *L. plantarum* NC8, in which not only plantaricin itself but also plantaricin-like peptides produced by other Gram-positive bacteria were shown to induce the production of plantaricin by *L. plantarum.* Fermented milk prepared by culturing the probiotic culture *Leuconostoc mesenteroides*, is a nutritious drink but at the same time a large number of spoilage microorganisms can grow in it when stored for a long time. The spoilage bacterial cultures are known to communicate with each other by release of signaling molecule which is described as quorum sensing. In a study of fermented milk, *Pseudomonas* was found to be responsible for spoiling the fermented milk. The signal molecule was identified as hexanoyl homoserine lactone. Two furanones namely 2(5H)-furanone and bromofuranone reduced acyl homoserine lactone and the shelf life was increased up to 9 days by reducing the spoilage bacterial cultures (Shobharani and Aggarwal, 2010).

Meat: Jay *et.al.,* in 2003 have reported the involvement of quorum sensing in the spoilage process of fresh meat products stored under aerobic refrigerated conditions and in the slime

formed on the meat surfaces. AHL signals, such as C4-HSL, 3-oxo-C6-HSL, C6-HSL, C8-HSL, and C12-HSL, have been detected in aerobically chill-stored ground beef and chicken by the members of *Pseudomonadaceae* and *Enterobacteriaceae* (Liu *et al.,* 2006). Additionally, food additives such as sodium propionate, sodium benzoate, sodium acetate and sodium nitrate may influence AI-2 production (Lu *et al*., 2004). In a study, Nychas *et al*., (2009) found that cell-free meat extract derived from spoiled minced pork meat stored aerobically at 5 and 20 °C contained QS signals. It was also observed, that the addition of cell-free meat extract from spoiled meat (containing QS signal molecules) to cultures of *Pseud. fluorescens* and *Ser.* marcescens resulted in an extension of the lag phase of *Pseud. fluorescens* but not of *Ser. marcescens* when compared to the control samples and in an increase of the metabolic activity for both the strains. The observed increase in metabolic activity was suggested to be related to the presence of some compounds in cell-free meat extract, including QS signal molecules (Nychas *et al*., 2009).

Fruits and vegetables: *Erwinia* and *Pseudomonas* produce various pectinolytic enzymes, namely, pectin lyases, pectate lyase, polygalacturonase, and pectin methyl esterases, which are responsible for the spoilage of ready-to-eat vegetables, also produce a broad range of AHLs (mainly 3-oxo-C6-HSL and C6-HSL). Moreover, inoculation of bean sprouts with AHL-producing pectinolytic *Pectobacterium carotovorum* increased the rate of its spoilage (Rasch *et al*., 2005). The pectinolytic activity of *Pseudomonadaceae* or *Enterobacteriaceae* (mostly *Erwinia* spp.) growing to high-cell densities (108 to 109 CFU g/L) in fruits and vegetables causes enzymatic browning, off-tastes, off-odors, and/or texture breakdown resulting in their spoilage. The pectinase of *Erwinia carotovora* is regulated by acylated homoserine lactone (Pirhonen *et al*., 1993), suggesting that rot is controlled by a quorum-sensing mechanism. In *S. marcescens* and *S. liquefaciens* secretion of several unrelated and potentially food-quality-relevant proteins such as the lipase LipA, the metalloprotease PrtA and the surface-layer protein (S-layer) SlaA (Riedel *et al*., 2001) is guided by QS.

In biofilms

Food technologists and hygienists globally face the problem of presence of biofilms on foods and food contact surfaces, food processing environments including drains and floors, on fruits, vegetables, meat surfaces, and in low-acid dairy products often causing concerns for food safety. A defining feature of many biofilm-forming bacteria is the secretion of extracellular polymeric substances (EPS). The formation of biofilms begin with bacteria attaching on the surface, cell-to-cell aggregation and proliferation, exopolysaccharide matrix production,

growth, maturation, and, finally, biofilm detachment or degradation. Quorum-sensing systems appear to be involved in all phases of biofilm formation. Many species, including the pathogen *Pseudomonas aeruginosa*, activate EPS production at high cell density (Hammer and Bassler, 2007). The foodborne pathogens *E. coli* O157:H7, *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Campylobacter jejuni* are known to form biofilms on food surfaces and food contact equipment, leading to serious health problems, and economic losses (Kumar and Anand ,1998). Cells embedded in a biofilm are more resistant to cleaning agents and other antimicrobial substances, making them difficult to eradicate from processing equipment (Stoodley *et al.*, 2002*). Listeria monocytogenes* biofilms were more resistant to cleaning agents and disinfectants including trisodium phosphate, chlorine, ozone, hydrogen peroxide, peracetic acid (PAA) and quaternary ammonium compounds (reviewed extensively by van Houdt *et al.*, 2007). Bacteria in bioûlms show increased resistance towards various organic acids, ethanol and sodium hypochlorite. Resistance is attributed to different mechanisms: a slow or incomplete penetration of the biocide into the biofilm, an altered physiology of the biofilm cells, expression of an adaptive stress response by some cells, or differentiation of a small subpopulation of cells into persisting cells. Biofilms on processing equipment surface are a source of contamination for food products due to the continuous detachment of cells and spores from the biofilm. Moreover, biofilm formation may cause economic losses due to equipment failure or necessary extensive cleanup (Kumar and Anand, 1998). Microorganisms in bioûlms catalyze chemical and biological reactions causing metal corrosion in pipelines and tanks, and they can reduce the heat transfer efûcacy if bioûlms become sufûciently thick at plate heat exchangers and pipelines (Mittelman, 1998; Vieira *et al.*, 1993). Most of the disinfectants used in cleaning are effective on free moving or planktonic bacteria. However, their effectiveness may decrease or may not be effective against bioûlm cells. If a microbial population faces high concentrations of an antimicrobial product, susceptible cells will be inactivated. However, some cells natural resistance or may acquire it mutation or genetic exchange. Such resistant bacteria can get into food contact surfaces and sometimes get into food too. Thus, there is a need to develop new control strategies especially the green strategies against biofilms. New control strategies are constantly emerging with main incidence in the use of biosolutions (enzymes, phages, interspecies interactions and antimicrobial molecules from microbial origin). Two questions need yet to be answered. (I) What parameters of a biofilm community inûuence the onset of quorum sensing (2) What are the functional consequences of quorum sensing in a bioûlm community.

In probiotics

The probiotic research conducted over the past 20 years has resulted in a valuable source of data related to health beneficial effects of probiotics which are viable bacteria which when administered in adequate amounts confer to a health benefit to the host. Latest explanation of the beneficial effects of probiotics is quorum sensing. Quorum sensing regulates the virulence expression in probiotics which may interfere with the signalling system avoiding the onset of virulence in pathogenic bacteria. Symbiotic gut microorganisms release various soluble low molecular weight molecules of different chemical nature (surface and exogenous proteins, nucleases, lectins, peptides, amines, bacteriocines, fatty and amino acids, lactones, furanons, etc.). These molecules are able to sense environment, interact with corresponding cell surface, membrane, cytoplasm and nucleic acid receptors, to reply quickly and coordinately by induction of special sets of genes, to support stability of host genome and microbiome. General scheme of the intestinal bacterium-eucaryotic cells "cross-talk" can be summerized here :

- A quorum-sensing molecule is released by a bacterial population into the intestinal lumen, and is directly active on the intestinal epithelium.

- Interactions between bacteria and the host cell surface require a direct contact.

- "Modulins" are soluble factors produced by the bacteria that act directly on host cell functions.

- Host cell biochemical pathways are specifically modified by these "modulins",

- Glycosylation modifications have an effect on the intestinal bacteria and on the intestinal epithelium defense mechanisms that help to fight against pathogens or virus infections (Freitas *et al.*, 2003).

Further studies have nicely shown that several gut properties were primarily dependent on the cross talk between epithelium and bacteria (Lopez Boado *et al.*, 2000). Probiotic *L. acidophilus* La-5 produces lactacin B when it senses live bacteria and the bacteriocin expression is controlled by an auto-induction mechanism involving the secreted peptide IP_1800 (Tabasco *et al.*, 2009). In probiosies, the luxS gene appears to have a clear role in acidic stress response in probiotic lactobacilli easing their survival in gut (Moslehi-Jenabian *et al.*, 2009). A recent report describes a bacterium isolated from the intestine gut of fish based on quorum sensing which has a probiotic characteristics by effectively reducing the amount of AHLs and the extracellular proteases activity of pathogen *Aeromonas hydrophila* (Chu *et al.*, 2011)
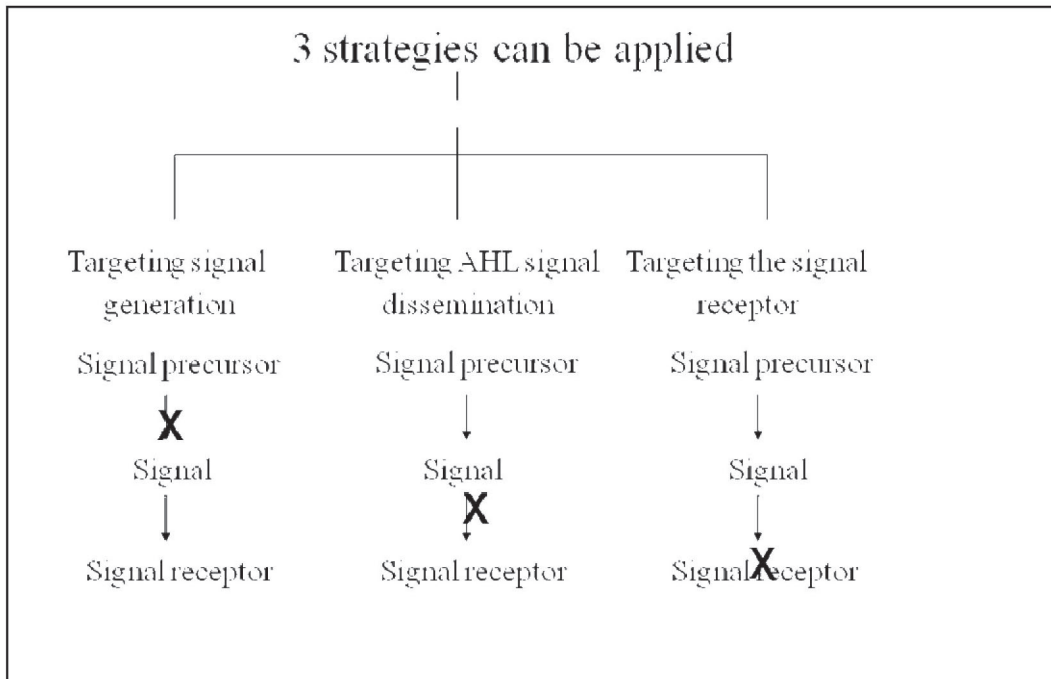
Figure 4: Strategies for quorum quenching

Gagging bacteria...... Quorum Quenching

Interference with bacterial cell-to-cell signaling via the quorum-sensing pathway to inhibit bacterial virulence and/or the development of biofilms is a new field of study with great potential applications.

QS inhibitors are not involved in bacterial growth, inhibition of QS should not yield a strong selective pressure for development of resistance. A few examples of QSI are mentioned here. QSI, C-30, a derivative of a natural furanone was shown to inhibit QS and cause *Pseudomonas* biofilms to be susceptible to clearance by detergent and antibiotics when the biofilms were grown in its presence (Hentzer *et al*, 2003). Peterson *et al* (2008) recently showed that Apolipoprotein B, the major structural protein of lipoproteins, sequesters the QS signal AIP1, consequently inhibiting Agr-dependent virulence in MRSA isolates. Rasko *et al* (2008) carried out a HTS to identify a lead structure (*N*-phenyl-4-[[(phenylamino)thioxomethyl]amino]- benzenesulfonamide) LED209 which selectively blocked binding of signals (AI-3/ epinephrine and NE) to QseC, preventing QseC's autophosphorylation, and consequently inhibiting QseC-mediated activation of virulence gene expression in EHEC O157. A remarkable study revealed by real-time RT-qPCR that the inhibition of virulence factors

regulation mechanisms by soluble molecules secreted by probiotics could represent an interesting way pathogenicity and virulence attenuation in *Ps. aeruginosa* nosocomial strains (Cotar *et al*., 2010). The anti-QS strategies developed so far have not yet been applied in a broad scale clinical trial and hence, it is difficult to assess their true potential and drawbacks at this stage. It is however certain that we need to expand our anti-microbial/anti-virulence targets and strategies. Pharmacologic interference of intercellular signaling can be envisioned at several steps in the quorum-sensing circuitry. Potential strategies include inhibiting receptor synthesis or function, reducing production or release of functional autoinducer, stimulating autoinducer degradation, or inhibiting autoinducer-receptor binding(Figure 4).

In a study by Vattem and others (2007), bioactive dietary phytochemical extracts from common dietary fruit, herb, and spice extracts significantly inhibited quorum sensing in *Chromobacterium violaceum*. The extracts also inhibited swarming motility in pathogens EC O157:H7 and *P. aeruginosa* (PA-01), known to be modulated by quorum sensing. Vanilla, a widely used spice and flavour, can inhibit bacterial quorum sensing. Girennavar *et al*., (2008) have reported that natural furocoumarins from grapefruit juice act as the potent inhibitors of AI-1 and AI-2 activity and biofilm formation in *S. typhimurium*, EC, and *P. aeruginosa*. These results suggest that grapefruit juice can serve as a safe source of alternatives

to the halogenated furanones to develop strategies targeted at microbial quorum sensing. Thus, quorum-sensing inhibitors can also be used as food preservatives in selected foods where AHL-regulated traits are responsible for food spoilage (Bai and Rai, 2011).

Biocontrol strategies that exploit bacterial QS provide an opportunity to

- Down regulate microbial activity and increase shelf life

- Alter microbial activity such that survival of the targeted microorganism is unlikely.

The main advantages with this approach is:

- Does not leave open an opportunity for other undesirable microorganisms to colonize the niche

- Down regulates the expression of enzymes such as proteases, thus limiting damage to the food.

- Utilizes the bacteria's own QS system against itself, thus decreases the possibility that the bacteria will adapt and become resistant to the QS inhibitor used.

Conclusion

Far from being singular entities, it is now apparent that bacteria exist in multifaceted communities and are constantly communicating with each other. Microbes cooperate for nourishment, movement, virulence, iron acquisition, protection, quorum sensing, and production of multicellular biofilms or fruiting bodies. The study of bacterial quorum sensing has suggested several ideal targets for drug design as well as agricultural and food industrial applications. Food spoilage is the outcome of the biochemical activity of a microbial community attributed to quorum sensing (QS). Consequently, if QS is extensively elucidated it would be a futuristic tool in designing approaches for manipulating these communication systems, thereby reducing or preventing, for instance, spoilage reactions or even controlling the expression of virulence factors. The QS can be exploited for the benefit of food preservation and food safety. Thus, the communication between bacteria can be used as a medium for enhancing bacterial performance in food systems like fermentation. Conversely in actions where bacterial crosstalks lead to spoilage and biofilms, targeting the signaling molecules would be one of the "green" or "clean label" approach that is the need of the hour.

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