Silent Signals

How Bacterial Communication Shapes Biofilms

C. Johnson, MS

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Biofilm Quorum Sensing and Its Impact on Food Quality and Safety

C. Johnson, MS

Quorum sensing (QS) is a critical bacterial communication mechanism that regulates the formation, maintenance, and resilience of biofilms that consist of bacterial communities encased in an extracellular polymeric substance (EPS) matrix and present significant challenges to food safety and quality. The EPS matrix not only protects biofilm bacteria from environmental stressors, such as chemical sanitizers, but also allows biofilms to adhere tightly to surfaces, making them extremely difficult to remove.

This paper examines the role of QS in biofilm formation and bacterial resistance, highlighting how QS enables bacteria to coordinate collective behaviors like virulence and biofilm development. As bacterial populations grow, the concentration of signaling molecules, or autoinducers, increases, triggering a synchronized response among the bacterial community to fortify the biofilm or produce protective substances against threats.

Given the persistence of QS-regulated biofilms, the paper discusses strategies for controlling biofilm formation, including the use of quorum sensing inhibitors (QSIs) and EPS-degrading enzymes. Quorum Sensing Inhibitors have shown promise in disrupting bacterial communication, thereby preventing biofilm formation and reducing pathogenicity. Research on QSIs is ongoing, and the potential of QSIs to enhance food safety by targeting QS pathways is significant.

However, challenges remain, such as the risk of bacteria developing resistance to QSIs and the need for regulatory approval. In the absence of widespread QSI application, vigilant process monitoring is essential for managing biofilm-related risks. Advanced monitoring technologies, like aseptic sampling systems, enable early detection and timely intervention to prevent contamination.

Understanding QS and its role in biofilm formation is vital for improving food safety and quality in food and dairy processing environments. While QSIs represent a promising future direction, integrating robust monitoring practices with existing sanitization protocols offers the best current approach to mitigating biofilm risks.

Introduction

Maintaining product safety and quality is paramount in the highly regulated environments of food and dairy processing. A significant challenge in these industries is the persistent threat posed by microbial biofilms, which can form on processing equipment and other surfaces. These biofilms, consisting of communities of bacteria embedded within a protective matrix, are difficult to eradicate and serve as reservoirs for pathogenic and product spoilage organisms. This can lead to contamination of food products, posing serious risks to consumer health and potentially leading to significant economic losses due to recalls and spoilage.

One of the critical factors enabling the formation, maintenance, and resistance of biofilms in food and dairy processing environments is quorum sensing (QS). Quorum sensing is a sophisticated bacterial communication system that regulates an array of collective behaviors, including biofilm formation, virulence, and resistance to environmental stressors such as heat and cleaning agents. Understanding the mechanisms of QS and its role in biofilm development is crucial for developing effective strategies to control microbial contamination in food and dairy processing. This report delves into the intricacies of QS, explores its impact on food quality and safety, and discusses potential applications of quorum sensing inhibitors (QSIs) as a novel approach to enhancing sanitation and safety in food and dairy processing.[1,2]

Mechanism of Quorum Sensing

Autoinducers and Signal Transduction

The core of QS lies in the production, release, and detection of chemical signaling molecules known as autoinducers. As the bacterial population grows, the concentration of autoinducers in the environment increases. Once a critical threshold concentration is reached, these molecules bind to specific receptors on bacterial cells, triggering a cascade of gene expression changes within the bacteria. Binding is the key element as it signals to the bacteria that they are now in a densely populated environment. Changes induced by gene expression are coordinated across the population, meaning many bacteria in the biofilm start doing the same thing at the same time.[3]

While it may appear that cells are randomly releasing autoinducers and hoping others pick them up, in reality, the process is very strategic. Bacteria produce autoinducers constantly in response to environmental triggers, and the accumulation of autoinducers is a natural consequence of growth. When enough bacteria are present, the autoinducers reach the necessary concentration to activate a collective response. This ensures that energy and resources are used efficiently—bacteria only start forming a biofilm when the local concentration at the initial attachment site reaches the necessary concentration of autoinducers to initiate biofilm formation. Even a small cluster of bacteria, if in

close proximity, can reach the necessary concentration of autoinducers to initiate biofilm formation. When the threshold is reached, all the bacteria in the group "know" it's time to start forming a biofilm or to increase the production of protective substances.

Here's how it works. Let's say a biofilm is exposed to an antibiotic or sanitizing agent, such as a reactive oxygen species (ROS). Some bacteria in the biofilm directly sense the toxic chemical and produce a small amount of an autoinducer specific to the toxic stress. As more bacteria detect the toxin, the concentration of this specific autoinducer increases, signaling to the entire community that a toxin is present.

Once the autoinducer level reaches a threshold concentration, it binds to receptors on the bacteria, triggering the activation of genes related to toxin resistance. These might include genes that produce enzymes to degrade the toxic chemical, efflux pumps to remove the toxin from the cells, or some other mechanism. The entire biofilm now starts producing these defenses simultaneously, making the bacterial community more resistant to this toxic stress.

Bacteria often have multiple QS systems specific to different environmental conditions or types of stress. Each system typically involves its own set of autoinducers and receptors tuned to respond to specific signals or threats. This allows bacteria to "fine-tune" their response based on the type of stress they encounter.

There are several types of autoinducers, and the specific molecules used can vary between bacterial species (Figure 1):

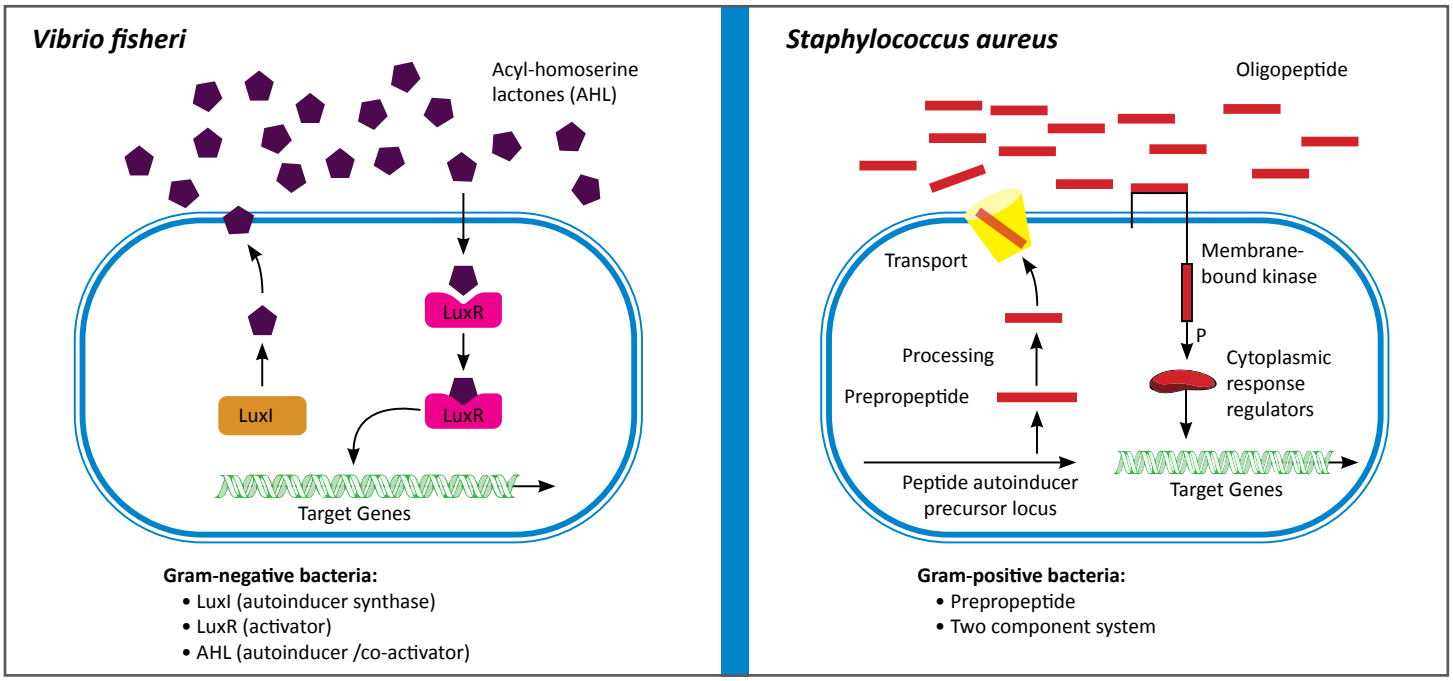
1. **Acyl-Homoserine Lactones (AHLs):** Commonly used by Gram-negative bacteria, AHLs are synthesized by the enzyme LuxI and are detected by the LuxR receptor. When the concentration of AHLs reaches a certain level, they bind to LuxR, forming a complex that activates or represses the transcription of target genes.[2]
2. **Oligopeptides:** In Gram-positive bacteria, QS often involves small peptides used as autoinducers and signaling molecules. These peptides (prepropeptides) are processed by the cell to become functional and are then secreted by the bacteria. Upon reaching a specific concentration, they are detected by two-component signal transduction systems. These systems typically involve a membrane-bound sensor kinase that binds with the peptide and phosphorylates a cytoplasmic response regulator that then modulates gene expression.[3]
3. **Autoinducer-2 (AI-2):** AI-2 is a signaling molecule used by both Gram-negative and Gram-positive bacteria, allowing for interspecies communication. AI-2 is detected by a sensor kinase that activates a signaling cascade that leads to changes in gene expression.[2]

Information Processing within the Cell

Once an autoinducer binds to its receptor, the receptor undergoes a conformational change that initiates a signal transduction cascade. This often involves the phosphorylation of a series of proteins, ultimately activating a transcription factor. This transcription factor then binds to specific DNA or RNA sequences in the promoter regions of target genes, either activating or repressing their transcription.[1]

For example, in the well-characterized LuxR/LuxI system of *Vibrio fischeri*, the LuxR-autoinducer complex binds to the promoter of the lux operon, leading to the production of luciferase and the emission of bioluminescence.[3] Similarly, in pathogenic bacteria such as *Pseudomonas aeruginosa*, QS regulates the expression of genes involved in virulence, biofilm formation, and antibiotic resistance.[4]

Figure 1



Adapted from Geethanjali, et al. 2018.17

Sanitation resistant bacteria in a biofilm. A biofilm is a community of bacteria where they acquire resistance to environmental stress, such as chemical sanitizers, and communicate with each other by quorum sensing.

Quorum Sensing and Extracellular Polymeric Substance Production

One of the critical roles of QS is regulating the production of extracellular polymeric substances (EPS), which is essential for biofilm formation and stability. Extracellular polymeric substances are a complex mixture of polysaccharides, proteins, lipids, and nucleic acids that forms the structural matrix of biofilms and allows them to adhere to surfaces and to each other, forming a structured community. This matrix also protects the bacteria from environmental stress, like changes in pH, temperature, or the presence of sanitizers.[5]

Regulation of EPS Synthesis

Quorum sensing controls the expression of genes involved in the synthesis of EPS components. For instance, in *Pseudomonas aeruginosa*, the QS systems las and rhl regulate the production of alginate, an essential polysaccharide in the biofilm matrix.[4] The las system, through the LasR-AHL complex, activates the expression of genes involved in alginate biosynthesis, while the rhl system modulates the production of rhamnolipids, biosurfactants that contribute to biofilm architecture.[5]

Similarly, in *Vibrio cholerae*, QS negatively regulates the production of Vibrio polysaccharide (VPS), a major component of the biofilm matrix. At low cell densities, when QS is inactive, the expression of VPS biosynthesis genes is upregulated, promoting biofilm formation.[2] As the population grows and autoinducer levels increase, QS activation leads to the repression of VPS production and the dispersal of the biofilm.[6]

Impact on Biofilm Formation

The production of EPS is crucial for the initial attachment of bacteria to surfaces and the subsequent development of a mature biofilm. The EPS matrix not only provides structural integrity but also creates a microenvironment that protects the embedded bacteria from environmental stressors, such as desiccation, UV radiation, and antimicrobial agents.[5]

In food processing environments, the formation of biofilms on equipment surfaces is a significant concern. Biofilms can develop on a variety of surfaces, including stainless steel, plastic, and rubber, and are notoriously difficult to remove once established.[7] The EPS matrix can bind and sequester nutrients, allowing the biofilm to persist even in nutrient-poor conditions. Additionally, the matrix can trap and concentrate enzymes and antimicrobial agents produced by the bacteria, further enhancing the biofilm's resistance to cleaning and sanitization efforts.[1]

Quorum Sensing and Resistance to Environmental Stress

Quorum sensing plays a vital role in enhancing the resistance of bacterial communities to environmental stressors, which is particularly relevant in food safety and quality. It ensures that biofilms are more than just a random collection of bacteria. It helps them function as a well-organized community. The bacteria in a biofilm are more resistant to environmental challenges because they are encased in a protective matrix and can quickly adapt to changing conditions by switching on stress response genes. The EPS matrix itself also acts as a barrier, making it harder for substances harmful to the biofilm to penetrate and reach the bacteria inside.[8]

Antibiotic Resistance

One of the most well-documented effects of QS is the regulation of antibiotic resistance genes.[9] In biofilms, QS can upregulate the expression of efflux pumps, which actively transport antibiotics out of the bacterial cells, reducing their intracellular concentrations and thereby conferring resistance.[6] Additionally, QS can induce the production of enzymes such as β-lactamases, which degrade antibiotics before they can reach their targets.[4]

The protective environment provided by the EPS matrix, combined with the QS-regulated expression of resistance mechanisms, makes biofilms highly resistant to antibiotic treatment.[5]

Resistance to Disinfectants and Sanitizers

Quorum sensing also contributes to the resistance of biofilms to disinfectants and sanitizers commonly used in food

processing facilities.[8] The EPS matrix acts as a physical barrier, limiting the penetration of these agents into the biofilm.[5] Moreover, QS can regulate the expression of genes involved in the neutralization of reactive oxygen species (ROS), which are often generated by disinfectants and sanitizers, and in the expression of efflux pumps that expel quaternary ammonium compounds from the cell.[10] This resistance is particularly problematic in food processing environments, where biofilms can harbor pathogenic bacteria that are difficult to eradicate, leading to persistent contamination and increased risk of foodborne illness.[7]

For example, in *Listeria monocytogenes*, a common foodborne pathogen, QS has been shown to regulate the expression of stress response genes that confer resistance to oxidative stress, including those encoding catalase and superoxide dismutase.[4] These enzymes detoxify ROS, allowing the bacteria to survive disinfectant exposure.

In *Pseudomonas aeruginosa*, QS controls rhamnolipid production, which can modulate the biofilm's surface hydrophobicity and influence the interaction with disinfectants.[8] The production of biosurfactants like rhamnolipids can also facilitate the detachment and dispersal of biofilm cells, potentially leading to the spread of contamination within the processing environment.[11]

Implications for Food Quality and Safety

Even in a constantly flowing environment, such as in fluid milk, liquid food, or beverage production lines, some bacteria can adhere to the surface of the piping, especially in areas where there is turbulence or the flow rate is lower (e.g., near bends or valves), or where there are surface irregularities (e.g., welds, gaskets, or crevices). In these instances, the role of QS in biofilm formation and resistance to environmental stressors has significant implications for food quality and safety.[7] Biofilms in food processing environments can lead to the contamination of food products, spoilage, and the spread of foodborne pathogens. The persistence of biofilms on processing equipment, despite routine cleaning and sanitization, poses a continual risk to food quality and safety.[9]

Contamination and Cross-Contamination

Biofilms can serve as reservoirs for pathogenic bacteria, which can intermittently detach and contaminate food products. This is particularly concerning in ready-to-eat (or drink) products, where post-processing contamination can lead to foodborne outbreaks.[7] Quorum sensing facilitates the formation of robust biofilms that are difficult to eliminate, increasing the likelihood of contamination.[8]

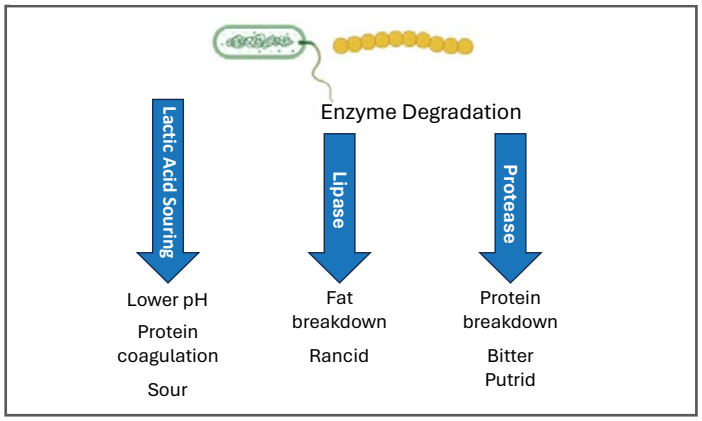
Cross-contamination is another critical issue, as biofilms on one piece of equipment can spread to other areas within the facility. For example, if biofilms form in a dead zone of a processing line, such as in a pipe junction or valve, the bacteria can be released during production and contaminate subsequent batches of food.[7] The ability of QS to regulate biofilm dispersal increases the risk of cross-contamination, especially when biofilms are disturbed during cleaning.[4]

Spoilage and Shelf Life Reduction

Quorum sensing can also contribute to food spoilage by regulating the production of spoilage enzymes and secondary metabolites.[12] In biofilms, QS can activate the expression of proteases, lipases, and other enzymes that degrade food components, leading to spoilage and off-flavors.[7] Additionally, QS-regulated secondary metabolites, such as volatile sulfur compounds, can produce undesirable odors and flavors in food products.[13]

The presence of biofilms on processing equipment can shorten the shelf life of food products by introducing spoilage organisms that continue to metabolize food components even after packaging.[14] This can lead to premature spoilage, economic losses, and decreased consumer confidence.

Mechanisms of Bacterial Spoilage



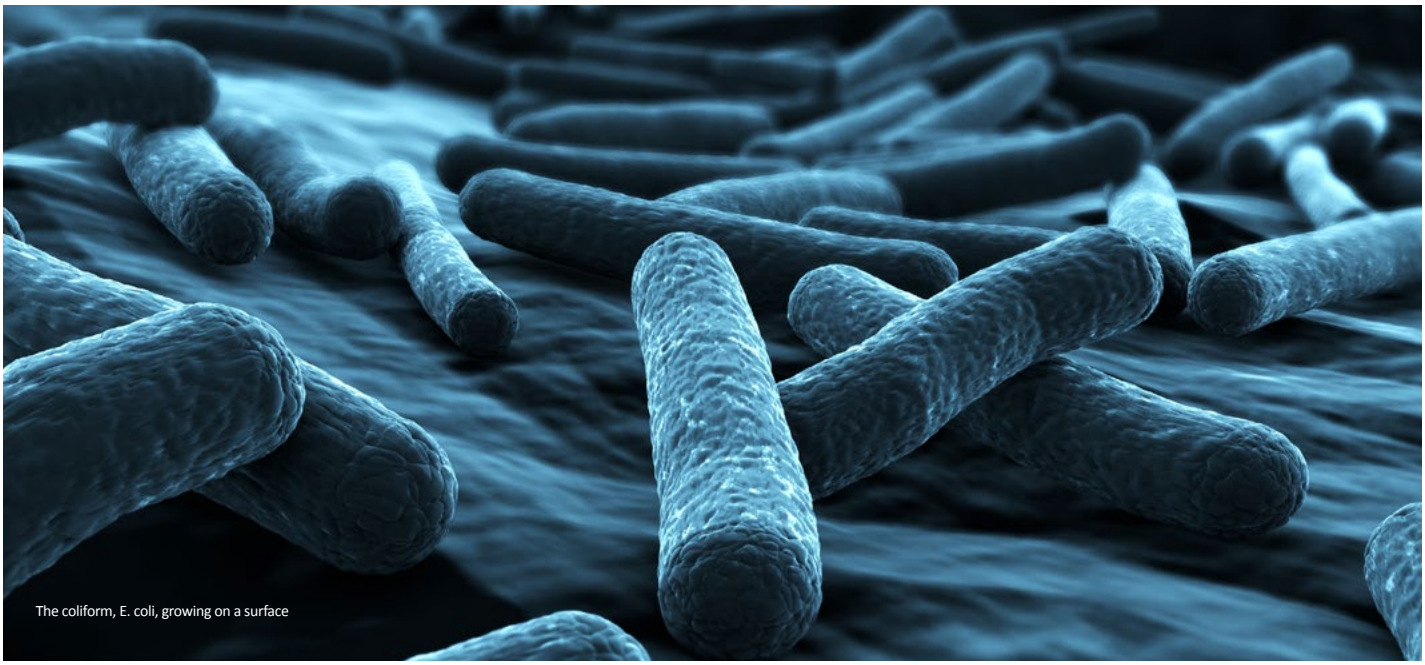
Bacteria use three mechanisms to spoil milk and dairy products: 1) Lactic acid souring; 2) breakdown of fats by lipase enzymes; and 3) breakdown of proteins by protease enzymes. Each causes characteristic flavor, odor, and textural change to the milk.

Challenges in Biofilm Control and Prevention

The complexity of QS systems and the resilience of biofilms present significant challenges for food processors. Traditional cleaning and sanitization methods are often insufficient to eradicate biofilms, necessitating the development of more effective control strategies.[8]

One approach being explored is using quorum sensing inhibitors (QSIs) to disrupt bacterial communication and prevent biofilm formation.[9] Quorum sensing inhibitors can block the synthesis or reception of autoinducers, inhibiting the coordinated expression of genes involved in biofilm development.[10] For example, furanones—naturally occurring compounds in some red algae—and halogenated furanones have been shown to interfere with AHL-based QS systems, reducing biofilm formation in *Pseudomonas aeruginosa* and other bacteria.[4]

Another strategy involves using enzymes that degrade the EPS matrix, such as DNases, proteases, and polysaccharide-degrading enzymes. By breaking down the structural components of the biofilm, these enzymes can enhance the penetration of disinfectants and facilitate the removal of biofilms from surfaces.[5]


The coliform, E. coli, growing on a surface

Integrating QSIs and EPS-degrading enzymes with traditional sanitization methods could improve biofilm control in food processing environments. However, the effectiveness of these approaches can vary depending on the specific QS systems and biofilm characteristics of the target bacteria.[7]

Applications of Quorum Sensing Inhibitors in Food Processing

Quorum sensing inhibitors represent a promising strategy for controlling biofilms and bacterial virulence in food processing environments. By disrupting the communication pathways that regulate these processes, QSIs can prevent biofilm formation, reduce the persistence of pathogens, and enhance the efficacy of cleaning and sanitization procedures. Research in this area is ongoing, and several potential applications and products are under development, with some already entering the market.[4,7]

Research on Quorum Sensing Inhibitors in Food Processing

Research on the use of QSIs in food processing is still in its early stages. Still, several studies have demonstrated the potential of these compounds to control biofilms and reduce contamination risks:

1. **Plant-Derived QSIs:** Many natural compounds, particularly those derived from plants, have been identified as potential QSIs. For example, furanones have been shown to inhibit QS in *Pseudomonas aeruginosa* and reduce biofilm formation.[4] These furanones mimic the structure of AHLs and competitively inhibit the binding of AHLs to their receptors, thereby preventing the activation of QS-regulated genes.
2. **Cinnamaldehyde and Vanillin:** Studies have shown that cinnamaldehyde (from cinnamon) and vanillin (from vanilla) can act as QSIs. Research by Nazzaro et al. (2013)[15] demonstrated that these compounds could inhibit biofilm formation by *Escherichia* coli and *Salmonella enterica* in food-related environments. Cinnamaldehyde has been found to interfere with the AI-2 signaling pathway, which many bacteria use for interspecies communication.
3. **Synthetic QSIs:** Synthetic QSIs, such as halogenated furanones, have also been studied for their ability to inhibit QS and biofilm formation. These compounds are designed to block the QS pathways more effectively than natural QSIs, offering a more robust approach to controlling biofilms.[10] For instance, studies have shown that synthetic halogenated furanones can prevent biofilm formation by *Listeria monocytogenes* on stainless steel surfaces, which is particularly relevant for food processing environments.[9]
4. **Enzyme-Based QS Disruption:** Enzymes that degrade autoinducers, such as AHL lactonases, have been explored as a strategy to disrupt QS. These enzymes break down the signaling molecules before they can activate QS pathways, thereby preventing the coordination of biofilm formation and virulence factor production. Research has shown that AHL lactonases can reduce biofilm formation by *Pseudomonas aeruginosa* and other pathogens on food processing surfaces.[16]

Quorum Sensing Inhibitor Products on the Market

Currently, few commercial products are specifically designed as QSIs for use in food processing environments. However, several natural antimicrobial agents with potential QS inhibitory effects are marketed for their ability to enhance food safety by reducing bacterial contamination and biofilm formation:

1. **Essential Oils:** Many essential oils, including oregano, thyme, and cinnamon oils, are marketed as natural antimicrobial agents. While their primary mode of action is not necessarily through QS inhibition, research has shown that components of

these oils can interfere with QS and reduce biofilm formation. For example, carvacrol, a component of oregano oil, has been demonstrated to inhibit QS in *Pseudomonas aeruginosa*.[15]

2. **Food-Grade QSIs:** Some companies are exploring the development of food-grade QSIs that can be used as additives in food processing or packaging to prevent bacterial growth. These products are still largely in the research and development phase, but they hold promise for enhancing food safety by targeting the communication systems that regulate biofilm formation.[7]

Prospects for Quorum Sensing Inhibitors in Food and Dairy Production

The future of QSIs in food and dairy production looks promising, but several challenges and opportunities need to be addressed:

1. **Regulatory Approval:** Regulatory approval is one of the main hurdles for the widespread adoption of QSIs in food processing. For QSIs to be used as food additives or on food contact surfaces, they must be thoroughly evaluated for safety and efficacy. Regulatory bodies such as the U.S. Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) will need to assess the potential impacts of QSIs on human health and the environment.[16]
2. **Combination with Traditional Sanitizers:** Quorum sensing inhibitors are likely to be most effective when combined with traditional sanitizers and cleaning agents. By disrupting quorum sensors, QSIs can weaken biofilms and make bacteria more susceptible to antimicrobial treatments. This combined approach could significantly enhance the effectiveness of cleaning protocols in food processing environments, reducing the risk of contamination and improving food quality and safety.[9]
3. **Development of Resistant Strains:** One concern with using QSIs is the potential development of resistance. Just as bacteria can develop resistance to antibiotics, there is a risk that they could evolve to bypass or overcome QSIs. To mitigate this risk, using QSIs in a targeted manner and in combination with other control strategies will be essential.[16]
4. **Incorporation into Food Packaging:** Another promising application of QSIs is their incorporation into food packaging materials. By embedding QSIs into packaging, it may be possible to inhibit QS and biofilm formation on food surfaces, extending shelf life and reducing spoilage. Research is ongoing to develop such packaging solutions that are both effective and safe for use in the food industry.[12]
5. **Targeting Specific Pathogens:** Future research may also focus on developing QSIs that target specific pathogens relevant to food safety, such as *Listeria monocytogenes*, *Salmonella enterica*, and *Escherichia coli O157*.[13] By targeting the QS systems of these pathogens, it may be possible to develop highly specific interventions that do not disrupt beneficial bacteria or the overall microbial ecology of food products.[10]
6. **Consumer Acceptance:** As with any new technology in food production, consumer acceptance will play a critical role in the success of QSIs. Transparent communication about the benefits and safety of QSIs will be essential to gaining consumer trust and ensuring the adoption of this technology in the food industry.[12]

The Importance of Vigilant Process Monitoring in the Absence of Quorum Sensing Inhibitors

While the potential for QSIs to disrupt bacterial communication and prevent biofilm formation offers promise for the future, these technologies are not yet viable for widespread application in the liquid food and dairy processing industries. Consequently, processors must rely on stringent and vigilant process monitoring practices as their primary defense against biofilm-related contamination.

In environments where bacteria can rapidly form and sustain biofilms, proactive monitoring becomes essential to maintaining hygiene and preventing contamination. Monitoring systems like the QualiTru TruStream aseptic sampling system provide critical insights into the microbiological status of liquid food and dairy processes. By enabling the regular and accurate collection of samples from key points within the processing line, the TruStream sampling system allows for the early detection of biofilm formation and microbial contamination before it can proliferate to harmful levels.

This approach is not just about detection; it's about prevention. The ability to monitor microbial activity at frequent intervals empowers processors to take timely corrective actions—whether through targeted cleaning, adjustments in processing parameters, or enhanced sanitization protocols. While no single strategy can completely eradicate the risk of biofilm formation, combining vigilant monitoring with effective cleaning and sanitization practices offers the best near-term solution for managing the threat of biofilms in food and dairy processing environments.

By focusing on process monitoring as a key prerequisite program (PRP), processors can mitigate the risks associated with biofilms, even in the absence of QSIs. The ongoing development and refinement of monitoring technologies will continue to play a pivotal role in enhancing food safety and quality, ensuring that biofilms remain a manageable challenge rather than a persistent threat.

Conclusion

Quorum sensing is a fundamental mechanism that regulates biofilm formation, EPS production, and resistance to environmental stressors in bacterial communities. In the context of food processing, QS contributes to the persistence and resilience of biofilms on equipment surfaces, posing significant challenges for food quality and safety. Understanding the intricacies of QS and its role in biofilm development is essential for developing effective strategies to control microbial contamination in food and dairy processing.

As research on QS and biofilm control continues to evolve, new insights and technologies will likely emerge, offering more targeted and efficient approaches to safeguarding food quality and safety. For now, integrating QSIs, EPS-degrading enzymes, and enhanced sanitization protocols represents a promising direction for mitigating the risks associated with biofilms in the food and dairy industries.

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