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Chapter

Nanosystems as Quorum Quenchers Targeting Foodborne Pathogens: Understanding the Inhibition Mechanisms and Their Docking Predictions

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Abstract

Food poisoning is one of the main problems affecting public health. Bacterial adhesion on surfaces has been documented for decades, and it is known that biofilm-forming bacteria are much more resistant than planktonic cells. Typically, nanosystems are studied regarding their antimicrobial activity (i.e., pathogenic bacteria such as *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus cereus*, and *Yersinia enterocolitica*), but not for antibiofilm activity and their associated genes. Some studies established protein-ligand prediction concerning quorum sensing suppression, commonly called quorum quenching. This chapter focuses on nanosystems or functionalized nanomaterials that have demonstrated antibiofilm or quorum quenching activity and, thus, establishes perspectives in modeling specific nanosystems to eradicate biofilms produced by foodborne pathogens.

Keywords: nanosystems, quorum quenchers, food safety, biofilm formation inhibition, molecular docking

1. Introduction

Foodborne diseases are a major public health concern, and one factor that has been shown to contribute to their persistence and virulence is biofilm formation by foodborne pathogens. Biofilms are complex structures composed of communities of

microorganisms that can attach to surfaces and resist antibiotics and other antimicrobial agents [1, 2].

Many foodborne pathogens, such as *S. aureus*, *Salmonella enterica*, *L. monocytogenes*, *E. coli* O157:H7, and *Vibrio*, can form biofilms. Biofilm formation by foodborne pathogens can occur in various settings, including food processing facilities and in the human body during infection. Their capacity to form biofilms can contribute to prolonged contamination of food processing surfaces, leading to outbreaks and significant economic losses. In addition, biofilms can provide a protective environment for the bacteria in the human body, making them difficult to eradicate and potentially leading to recurrent infections [3].

Efforts to control biofilm formation by foodborne pathogens are important for reducing the risk of foodborne disease. Strategies to prevent and mitigate biofilm formation include proper cleaning and disinfection of food processing surfaces, targeted use of antimicrobial agents, and development of new drugs that can disrupt biofilm formation.

1.1 Biofilm formation

The biofilm formation process differs among bacteria, but the process generally involves several stages [4]. Biofilms are formed by extracellular polymeric substances (EPS) that may be composed of proteins, DNA, and polysaccharides [5, 6]. Some bacteria can form this type of biofilm in situations of change or stress. A bacterial community is formed with the ability to adhere to inert materials or living tissues. In this microenvironment, the bacteria among themselves carry out a type of communication, or chemical signaling, that consists of the production of inducing molecules [communication by autoinducers (AI)]. This type of signaling mechanism is known as "quorum sensing.". Quorum sensing (QS) communication occurs not only between bacteria, but they can also associate a microorganism different from the initial species through the biofilm; the other microorganisms will have the same genes that they express for biofilm formation and thus be able to also resist the stress that initiated the mutation, as shown in **Figure 1** [7, 8].

Different structures and proteins are related to biofilm formation and stabilization, whether Gram-positive or -negative bacterium is involved. These include lipopolysaccharides, exopolysaccharides, QS, teichoic acids, and others [9, 10].

The specific genes associated with QS and biofilm formation can vary between foodborne pathogen species and strains; some examples are summarized in **Table 1**.

1.2 Quorum sensing and quorum quenchers

Quorum sensing, a process of cell-to-cell communication used by many bacteria to regulate their behavior, has been identified as a potential target for combating foodborne pathogens. Quorum quenchers (QQs) are compounds that can interfere with QS, representing a promising strategy to control bacterial infections. In recent years, the use of nanotechnology-based approaches, such as green synthesis of nanoparticles, has been explored for synthesizing alternative QQs agents that can be used against foodborne pathogens. Additionally, with the advent of computational chemistry and molecular docking techniques, it has become possible to develop computational models that can predict the interactions between QS and QQs.

The use of quorum quenchers has been an attractive approach to inhibit the virulence and biofilm formation of bacteria, without killing them, thus reducing the

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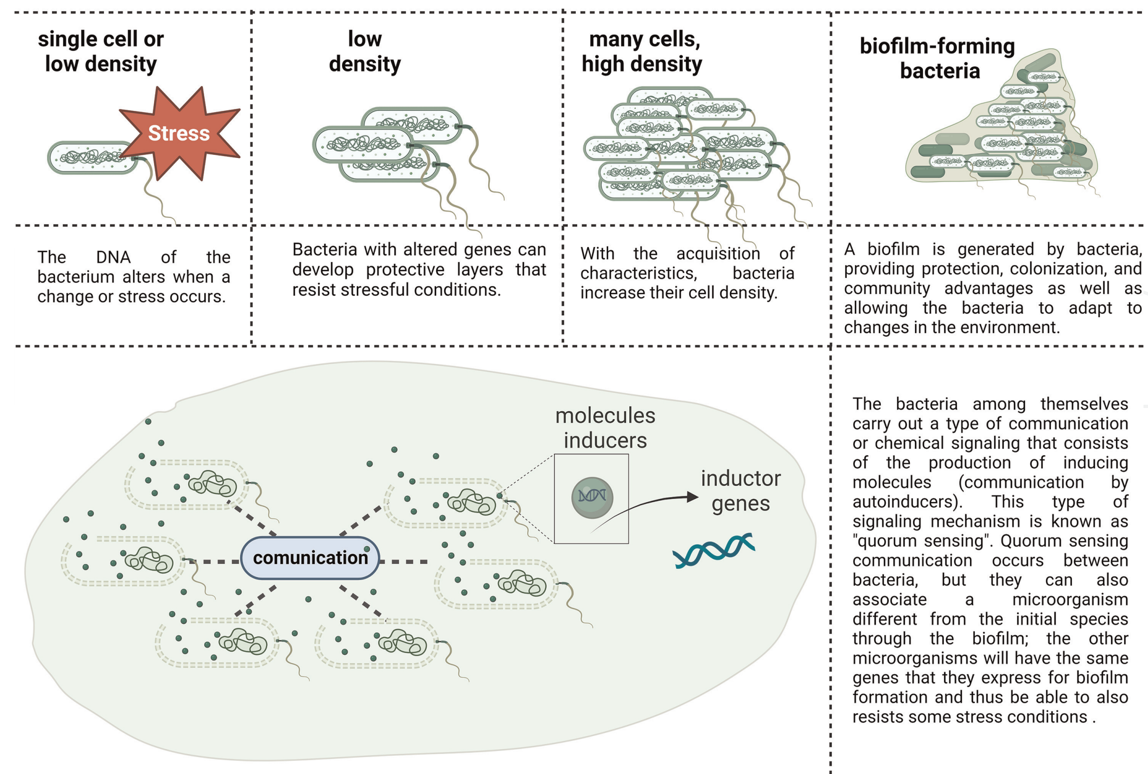

Figure 1.
Biofilm formation and quorum sensing bacteria.

Gene	Microorganism associated	Function
*lux*I and *lux*R	*Vibrio fischeri*, *E. coli*, *S. enterica*	Production and detection of quorum sensing signaling molecule acyl-homoserine lactone (AHL).
*las*I and *las*R	*P. aeruginosa*	Control production and detection of N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL).
*rhl*I and *rhl*R	*P. aeruginosa*	Control production and detection of N-butyryl-homoserine lactone (C4-HSL).
*agr*A and *agr*C	*S. aureus*	Control production and detection of autoinducing peptide (AIP).
*lux*S	Various	Production of autoinducer-2 (AI-2).
csg and *bcs*	*E. coli*, *S. enterica*, *L. monocytogenes*	Production of curli and cellulose, respectively, which are essential components of the biofilm matrix.
*rpo*S	*S. enterica*, *E. coli*	Encodes for the RpoS protein, involved in the regulation of stress response and the formation of persistent cells in biofilms.

Table 1.
Genes associated with quorum sensing in Gram-positive and negative bacteria [11, 12].

risk of resistance development. There are different types of QQs, such as enzymatic and non-enzymatic, based on different mechanisms of action:

1. *Enzymatic QQs*: These are enzymes that specifically degrade the signaling molecules that mediate QS. Examples include lactonases, acylases, and oxidoreductases. These enzymes can hydrolyze the acyl-homoserine lactones

(AHLs) or peptides, the most common QS molecules, into inactive forms that cannot activate the QS machinery.

2. *Non-enzymatic QQs*: These are small molecules that can bind to QS signaling molecules and prevent them from binding to their receptors or even degrade them. Examples of non-enzymatic QQs include furanones, halogenated furanones, or quinolones.

3. *Natural QQs*: Some phytocompounds or bacteria have evolved quorum quenching mechanisms that help them regulate interactions with other bacteria. These natural QQs comprise a complex mixture of compounds, such as essential oils, gingerol, kahweol, resveratrol, curcumin, and many others.

Quorum quenchers can be used in many ways against bacterial infections. For example, they can be used to prevent or disrupt biofilm formation on surfaces, such as medical equipment. This is especially important in hospital settings, where microbial biofilms on medical devices can be a major source of infection. QQs can also be used in combination with antibacterial agents to enhance their efficacy and reduce the risk of resistance development. For instance, using quorum quenchers to mitigate resistance development can be useful in chronic infections.

1.3 Nanosystems

Nanoparticles (NPs) are particles that have at least one dimension between 1 and 100 nanometers. They can be naturally occurring or artificially created and have unique physical and chemical properties that differ from those of their bulk counterparts. They are studied in many scientific fields such as physics, chemistry, geology, and biology. NPs have many applications in industry and medicine, such as in drug delivery systems, in targeted cancer therapies, and in developing new materials and electronics.

Antimicrobial nanoparticles are types of nanomaterials that have shown potential for use as antimicrobial agents due to their unique physicochemical properties. They can be synthesized from a variety of materials including metals, metal oxides, and polymers. These NPs can be used to inhibit the growth of different microorganisms such as bacteria, viruses, and fungi, either by disrupting their cell membranes or by interfering with their metabolic processes. Additionally, antimicrobial nanoparticles have been studied for use in applications such as food packaging, wound dressings, and water treatment.

Nanosystems in biological science typically refer to systems or structures at the nanoscale that are relevant to the study of biology. Nanosystems have emerged as a promising approach for the prevention of biofilm formation and antimicrobial resistance.

Nanosystems have several mechanisms that can prevent biofilm formation and overcome antimicrobial resistance. These mechanisms include the physical disruption of biofilm, targeting biofilm matrix, preventing bacterial adhesion, and releasing antimicrobial agents in a controlled and sustained manner. In addition, nanosystems can improve the pharmacokinetics and pharmacodynamics of antimicrobial agents, enhancing their efficacy and reducing their toxicity.

Nanosystems have also demonstrated great potential in the development of novel antimicrobial agents that can overcome antimicrobial resistance mechanisms. For

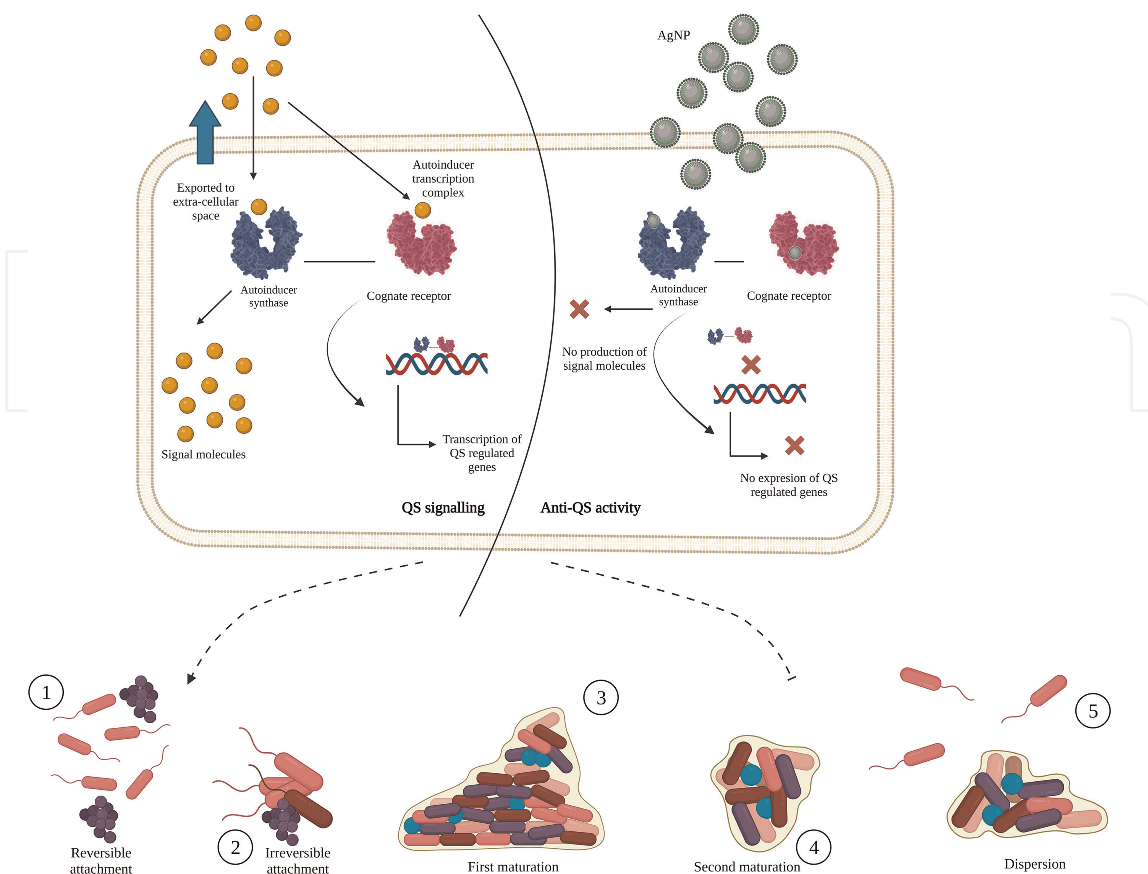

Figure 2.
AgNPs QS signalling and anti-QS mechanism in biofilm formation in bacteria.

example, the use of silver nanoparticles (Ag-NPs) has been reported to be effective against several drug-resistant bacteria, including methicillin-resistant *S. aureus* (MRSA) and *Pseudomonas aeruginosa*. In **Figure 2**, we can observe the mechanism by which QS and anti-QS activity occur in bacteria, leading to the formation (dashed arrow, left) or inhibition of biofilm (dashed line, right) throughout the five stages of the bacterial biofilm formation process.

However, the development of nanosystems as a viable alternative to traditional antibiotics requires careful consideration of their potential toxicity, biocompatibility, and long-term safety.

1.4 Molecular docking

Molecular docking is a computational method used to study the interactions between a protein and a small molecule, called a ligand. The goal is to predict how the ligand will bind to the protein and the strength of that interaction.

The process of molecular docking involves several steps, including preparing the protein and ligand structures, generating a docking grid, and running the docking simulation. The simulation predicts the optimal docking position and conformation, with the goal of optimizing a scoring function. After running the simulation, the result is analyzed to better understand the protein-ligand interactions and assess the potential binding strength. The result is then validated using experimental data or benchmarking.

Recently, molecular docking has been explored for its potential applications in biofilm formation control by targeting QS systems of bacteria. By understanding how small molecules interact with key components of QS pathways, we can develop strategies to disrupt these pathways and thus prevent biofilm formation without harming other beneficial bacterial species or human health concerns associated with antibiotic use.

In recent years, several studies have employed molecular docking as a tool for the design and development of novel antibiofilm agents. For example, a study by Khadke, see [13], used molecular docking to evaluate the binding affinity of cinnamaldehyde analogs with the biofilm response regulator yeast-form wall protein 1 (YWP1) and upregulated by cAMP in filamentous growth (UCF1) in *Candida albicans*. The study demonstrated that the cinnamaldehyde analogs could effectively inhibit biofilm formation in the yeast. Moreover, molecular docking can assist in identifying the interaction mechanism of an antibiofilm agent with its target molecule. For instance, a study by Ren [14] showed that isookanin could bind to biofilm-related proteins and interrupt biofilm formation in *Staphylococcus epidermis*.

For this, molecular docking is a promising technique for the design and development of novel antibiofilm agents. The use of molecular docking could assist in identifying the interaction mechanism of an agent with its target molecule and predict the therapeutic efficacy of the agent. However, further studies are necessary to validate the outcomes of molecular docking through *in vitro* and *in vivo* experiments.

2. *P. aeruginosa*

P. aeruginosa is an opportunistic Gram-negative bacterium associated with nosocomial diseases, mainly lung and airways infections. One of the main mechanisms of action relates to using degradation enzymes and biofilm formation. The biofilm produced by *P. aeruginosa* gives it a sessile behavior making it difficult to attack by antimicrobial agents due to its exopolysaccharide nature and persistence of virulence factors [15, 16].

The genus *Pseudomonas* spp. is involved in the colonization and management of processes in the dairy industry in which different temperatures are used, allowing to take advantage of these conditions and some food structures, as surface substrates for biofilm formation [1].

2.1 Quorum sensing and related genes

Bacterial growth of *P. aeruginosa* has shown structural and metabolic changes ranging from the planktonic phase to the sessile or cellular attachment phase, identifying up to five stages in which biofilm formation takes place. These stages are: (1) reversible attachment, (2) irreversible attachment, (3) first maturation, (4) second maturation, and (5) dispersion, where each of them exhibits the expression of different protein patterns [17, 18].

In Gram-negative bacteria, AHLs have been characterized as the main molecules triggering QS signaling. In the QS of *P. aeruginosa*, several genes associated with each of the stages of biofilm formation have been identified. Signaling and regulatory genes include: N-3-oxododecanoyl *L*-homoserine lactone (3-oxo-C12-HSL), N-butyryl-homoserine lactone (C4-HSL), 2-heptyl-3-hydroxy-4-quinolone, and 2-(2-hydroxyphenyl)thiazole-4-carbaldehyde, controlled by the regulatory systems

such as *las*I/R, *rhl*I/R, *pqs*ABCDE/*pqs*R, and *Amb*BCDE/*Iqs*R, respectively. The synthesis of these AI improves protein expression leading to an increase of factors involved in QS. Also, maturation of the biofilm is regulated by this feature, following irreversible attachment, production of virulence factors involved in *P. aeruginosa* pathogenicity, iron scavenging activity, motility, and dissemination [19, 20].

Biosynthesis inhibition of AI molecules can be biodirected through modified nanosystems as an alternative to diminish the virulence of *P. aeruginosa*.

2.2 Nanosystems tested *in vitro* and *in silico* for biofilm inhibition

In effort to inhibit QS mechanisms of opportunistic bacteria like *P. aeruginosa*, green alternatives based on nanosystems have been developed. **Table 2** exhibits nanosystems synthesized using plant extracts, microorganisms, or bioactive coatings, aiming to inhibit biofilm formation and QS activity of *P. aeruginosa*. In 2017 [24], Elshaer and Shaaban proposed selenium nanoparticles (Se-NPs) coated with honey polyphenols used as nanovectors in drug delivery systems, suggesting molecular docking studies as demonstration of antivirulence potential against *P. aeruginosa*.

Additionally, antibiofilm activity of 68% was exposed for Au-NPs, with a quorum quencher activity up to 88% at 4.6 $\mu g \cdot mL^{-1}$, using synthesis of NPs by biological reduction with different strains of *Streptomyces* isolated from soil. The reduction of selenium and gold metal ions to nanometals (Se-NPs and Au-NPs) was carried out

Nanosystem	Antibiofilm activity assay %	Quorum quencher activity (% or concentration)	Ligands	Receptor protein (genes)	Reference
Se-NPs Au-NPs	64–88% with 2.3 $\mu g \cdot mL^{-1}$ 26–68% with 4.6 $\mu g \cdot mL^{-1}$	65–90% with 2.3 $\mu g \cdot mL^{-1}$ 40–88% with 4.6 $\mu g \cdot mL^{-1}$	Pyocyanin Elastase Protease	*las*R *las*I *rhl*I *rhl*R *pqs*A *pqs*R *tox*A *las*A *las*B	[21]
ZnO nanospikes	20–85% with 25–200 $\mu g \cdot mL^{-1}$	35–75% with 25–200 $\mu g \cdot mL^{-1}$	Elastase Protease Pyocyanin	*las*B	[22]
Piper betle-Ag-NPs	78.20% with 8 $\mu g \cdot mL^{-1}$	82.43% with $\mu g \cdot mL^{-1}$	Eugenol Pyocyanin Elastase	*las*I *las*R *Mvf*R	[23]
Se-nanovectors coated with honey	>90% with 4.5 $\mu g \cdot mL^{-1}$	100% 4.5 $\mu g \cdot mL^{-1}$	Acacetin Apigenin Pinocembrin Pinobanksin Quercetin Caffeic acid Kaempferol	*las*R	[24]

Table 2.
Biofilm formation inhibitors nanosystems tested in vitro *and* in silico *against* P. aeruginosa.

under green chemical conditions monitoring the antibiofilm activity by crystal violet method [21]. The anti-QS and antibiofilm activity of zinc oxide (ZnO) nanospikes coated with cetyltrimethylammonium bromide (CTAB) was shown by Prateeksha [22], with different incubation times. The antibiofilm activity of *P. aeruginosa* was determined using the polyvinyl chloride biofilm formation assay and crystal violet cell attachment assay, in addition to elastase and protease transcriptional activity analysis for QS [22, 25]. About aqueous plant extracts, a bioreduction of Ag-NPs mediated by *Piper betle* (*Pb*) leaves was evaluated using molecular docking of the interaction of NPs conjugated with Eugenol (the main phenolic compound of *Pb* leaves) and QS-associated proteins of *P. aeruginosa* [23]. Results revealed an antibiofilm activity of 78% and a quorum quencher activity of 82% at 8 $\mu g \cdot mL^{-1}$ concentration, implying considerable interactions between Eugenol-Ag NPs and QS-regulatory proteins.

The characteristics steered by each of these biologically mediated nanosystems provide a perspective for green molecular strategies targeting microorganisms such as those developed in this chapter.

3. *Salmonella*

Salmonella species are a group of Gram-negative bacteria, which causes animal and human infections. *Salmonella* genus contains two species, *S. enterica* and *Salmonella bongori*, the first being subdivided into six subspecies [26]. Based on the clinical syndromes that *Salmonella* spp. cause, it could be classified as typhoid *Salmonella* and non-typhoid *Salmonella* (NTS). Only Typhi and Paratyphi serotypes are causative agents of typhoidal fever, an acute illness with symptoms that include high fever, malaise, and abdominal pain; typhoid fever has been associated with 600,000 deaths per year [27]. NTS serotypes cause gastroenteritis that is typically uncomplicated, however, it can be severe for immunosuppressed patients, elderly, and infants. NTS illnesses are the fourth morbidity and the third leading cause of deaths among diarrheal diseases worldwide. Every year, *Salmonella* spp. causes 93 million cases of gastroenteritis and more than 150,000 deaths, among these, 85% of cases were food linked [28–30]. The most prevalent serovars of *Salmonella* are Enteritidis, Newport, Typhimurium, and Javiana [31, 32].

3.1 Quorum sensing and related genes

During biofilm formation, microorganisms can communicate with each other through QS to regulate metabolic activity. QS is mediated by three mechanisms of autoinducers: AI-1, AI-2, and AI-3. The main regulators of pathogenic *Salmonella* are AI-2 and AI-3 [33].

Salmonella produces AI-2 through *luxS* gene, and SdiA protein detects AHLs produced by other bacterial species, with preference of oxoC18 modification; however, it can detect AHLs with other structures such as oxoC12 produced by *P. aeruginosa,* C4 produced by *Aeromonas hydrophila*, or C6 and oxoC6 produced by *Y. enterocolitica* [34, 35]. AI-2 signaling requires low pH and high osmolality, as low osmolality induces signal degradation. The AI-3 synthetic pathway and chemical formula remain unknown. Despite this, epinephrine, norepinephrine, and catecholamines are associated with AI-3 regulation [35].

Many genes are associated with *Salmonella* biofilm formation, like *lux*S/AI-2/*lux*R homolog SdiA system related to QS, the QseBC two-component system also associated with QS, and a universal regulator of virulence [34]. Other genes associated with

biofilm formation are *Mig-14* and *Virk* genes, which reduce outer membrane permeability and induce polymyxin B resistance, *Mig-14* gene is stimulated by antimicrobial peptides and acidic pH conditions [36]. *S. enteritidis* and *S. typhimurium* have the *rck* gene on virulence plasmids, inducing cellular adhesion and increasing bacterial resistance to serum [37].

3.2 Nanosystems tested *in vitro* and *in silico* for biofilm inhibition

Many strategies have been developed to achieve biofilm and QS inhibition, including *in vitro* and *in silico* tests. A virtual screening was performed by Almeida, see [38], for nonsteroidal anti-inflammatory drugs, searching potential inhibitors of QS in *Salmonella* using molecular docking. This study considered three different macromolecular arrangements of SdiA protein observing binding affinities testing more than 193 compounds. Z-phytol and lonazolac molecules were recognized as candidates for *in vitro* inhibition. Also, *in silico* anti-QS activities of Benzeneethanamine, 4-methoxy- and 2-Cyclopentadecanone, 2-hydroxy by a SdiA protein interaction were predicted [39]. Phytochemical berberine was studied [40] as an antibiofilm inhibitor using crystal violet microtiter plate assay; berberine showed 31.20% antibiofilm activity at 0.019 mg·mL^{-1} in front *S. enterica* sv Typhimurium and QS inhibitory potential was screened using Chromobacterium *violaceum* biosensor bacteria. Inhibitors of biofilm and Quorum quenchers are presented in **Table 3**, using plant-based molecules tested *in vitro* and *in silico*.

Nanosystem	Antibiofilm activity assay %	Quorum quencher activity (% or concentration)	Ligands	Receptor protein (genes)	Reference
Plant compounds and nonsteroidal anti-inflammatory drugs	Not specified, indicated as high activity in 83.20% of the compounds	†	193 compounds anti-inflammatory and plant compounds	SdiA (4Y13-S, 4Y15-S, 4Y17S)	[38]
Phytocompounds of *Psidium guajava*	†	†	Benzeneethanamine, 4-methoxy- and cyclopentadecanone, 2-hydroxy-	SdiA	[39]
Berberine	31.20% with 0.625 mg mL^{-1}	†	Berberine	*las*R	[40]
Lactic acid Malic acid	†	80.2% in spinach 76.6% in cantaloupe 46.7% in spinach 37.5% in cantaloupe 80.4% in cantaloupe	Lactic and malic organic acids	*lux*S	[41]
Ag-NPs of *Myristica fragrans* seed extract	87% nutmeg aqueous seed extract 99.1% with 50 μg·mL^{-1} Ag-NPs	†	6,6a-dihydro-1-(1,3-dioxolan-2-yl)-, (3aR, 1-t, octadecane, 6-methyl-, heptadecane, 2,6,10,14-tetramethyl-BIS (2-ethylhexyl) phthalate	RcsB RcsC	[42]

†Not declared.

Table 3.
Biofilm formation inhibitors nanosystems tested in vitro *and* in silico *against* Salmonella *spp.*

4. *E. coli*

E. coli is a Gram-negative, rod-shaped bacterium from the *Escherichia* genus. Different serotypes that produce toxins are associated with foodborne diseases, such as *E. coli* O157:H7, commonly known as enterohemorrhagic *E. coli* (EHEC). This pathogen is responsible for bloody diarrhea outbreaks and hemolytic uremic syndrome worldwide [43].

Virulence of EHEC is well studied, and it involves different mechanisms through which pathogen survives the acid environment in the stomach of the host, and colonizes the intestine, where lesions are provoked and, consequently, bloody diarrhea occurs [44].

4.1 Quorum sensing and related genes

Quorum sensing of *E. coli* is mediated by the *lux*I/*lux*R system, where AI are synthesized and recognized; as a result, different phenotypes, such as bioluminescence, antibiotic production, biofilm formation, and virulence factors' secretion, are expressed [45]. The most common AI in *E. coli* are AHLs, and some of these lactones have different lengths of acyl tail (4–20°C), oxidation at the third carbon in the acyl tail (carbonyl, alcohol, or methylene), units of unsaturation, or aryl located opposite to aliphatic tails [45, 46].

Another receptor found in *E. coli* is the SdiA receptor, for which the specific AHL is not present in the genome of this pathogen. Instead, this receptor can interact with AHLs produced by other bacteria that exist in the host, favoring the expression of the *gad* operon, which includes proteins associated with acid environment resistance, a crucial step in the colonization and infection of *E. coli*. In the intestine, SdiA-AHL complex is dissociated, leading to the activation of the LEE operon (locus of enterocyte effacement), which is related to lesions provoked on the walls of the intestine and bloody diarrhea [45].

The disruption of the QS has been one of the most studied methods to control growth and virulence of foodborne pathogens, and some authors suggest different pathways to achieve this disruption [46]:

- Inhibition of AI synthesis.

- Enzymatic degradation of AI.

- Use of materials that adsorb or quench AI.

- Use of compounds that mimic AI affinity for the proteins related to recognition of AI.

4.2 Nanosystems for QS inhibition

Inhibition of QS has gained more attention nowadays, since AHLs can go in and out of a cell, different molecules have been proposed to interact with the specific receptors, mimicking the AHL structure or blocking the receptors. In **Table 4**, some examples of QS inhibitors and their proposed mechanisms are compiled.

Nanosystem	Synthesis method	Concentration	Mechanism	Reference
Chitosan (CS)-NPs with quercetin	Ionotropic gelation	†	Quercetin binds to different domains of LuxR receptor, affecting binding affinity to LuxI-DNA. Other suggestion is that quercetin blocks the secretion of AHL to the cytosol.	[47]
CS Nanoemulsion/ Nanocapsule doped with cinnamaldehyde	Spontaneous emulsification	0.5 mM	Green fluorescence protein (GFP) inhibition.	[46]
D-limonene nanoemulsion	Spontaneous emulsification	†	AI synthesis and motility reduction, suppression of extracellular polymeric substances (biofilm formation inhibition).	[48]
CS-NPs dually crosslinked with genipin and sodium tripolyphosphate (TPP)	Ionotropic gelation	0.001–0.1 mg·mL^{-1}	AHL-mediated fluorescence response decrease.	[45]
CS nanosystem functionalized with mannose	Reductive amination reaction	3 mg·mL^{-1}	Interference with lectins, avoiding adhesion, motility, and biofilm formation.	[49]

†Not declared.

Table 4.
Nanosystems and their mechanisms of E. coli *QS inhibition.*

4.3 Molecular docking applied in *E. coli*

Several researches have modeled the possible interaction of nanosystems of bioactive compounds on specific genes or proteins expressed by *E. coli*. For example, synthetic thiazolo[3,2-α]pyrimidine molecules were obtained using ZnO nanoparticles as catalyst, and the *in silico* test was used to determine with which residues from the DNA gyrase B they bind. The results are shown in **Table 5**.

According to Schembri [51], several genes are affected in expression during biofilm formation, growth, and stationary phases. Among them, it has been shown that *flu* and *rpo*S are two of the most important genes for *E. coli* biofilm formation. *Flu* expresses the formation of the antigen 43 (Ag43), which deals with autoaggregation of cells, a primary step for biofilm to begin formation, while *rpo*S activates other genes in charge of dealing with stress conditions such as carbon starving, oxidative degradation of DNA, osmotic stress, etc.

Zinc oxide nanoparticles obtained with green synthesis using *Dysphania ambrosioides* extract were evaluated for molecular docking against *E. coli*. It was demonstrated that ZnO nanoparticles interact with the AcrAB-TolC proteins, which is a pump that crosses the inner and outer membrane of Gram-negative bacteria. This protein has been associated with resistance to antibiotics development, for which inhibiting its expression may lead to growth control of the pathogen [52].

In other cases, the extracts used in green synthesis of nanoparticles are studied for molecular docking. Such is the case of *Aegle marmelos* extract used to obtain copper oxide nanoparticles, and which was estimated to show that the major components

Molecule	Docking score (kcal·mol^{-1})	H-bonds	Amino acid residual interaction	
			Hydrophobic/ Pi-cation/ Pi-anion/ Pi-alkyl interactions	Van der Waals interaction
	−7.4	Asn-46, Thr-165	Val-43, Ala-47, Gly-77, Ile-78, Ile-94, Val-167	Asp-49, Glu-50, Asp-73, Arg-76, Pro-79
	−7.6	Gly-77, Thr-165	Val-43, Ala-47, Asp-73, Ile-78	Pro-79
	−7.4	Ser-121	Ile-78, Pro-79, Ile-94	Val-97, Leu-98, Gly-119, Val-120
	−7.3	Glu-50	Arg-76, Ile-78, Pro-79	Ala-47, Asp-73, Gly-77, Thr-165
	−7.6	Asn-46	Val-43, Ala-47, Asp-73, Gly-77, Ile-78, Ile-94, Thr-165, Val-167	Val-71, Val-120

Table 5.
Bound residues of E. coli *DNA gyrase B of synthetic thiazolo[3,2-α] pyrimidines [50].*

were beta-sitosterol, gamma-sitosterol, and marmesin. These compounds were studied *in silico* against the BamA protein of *E. coli*, a pump that allows substrates to insert into the outer membrane of Gram-negative bacteria. Results showed binding energies of −8 kcal·mol^{-1}, except for gamma-sitosterol (12 kcal·mol^{-1}). Interaction of these compounds present in the extract suggests good inhibition of the protein. Beta-sitosterol forms both polar and non-polar bonds like hydrogen, pi-sigma, pi-alkyl bonds, and Van der Waals interaction with the residues GLU435, PHE494, TYR653, and ASN666 of the BamA; on the other hand, gamma-sitosterol binds only to the ASN534 and TYR468. Both present a stable complex. Finally, marmesin binds with

ARG734, THR588, PHE586, and ARG583 with hydrogen bonds, while non-polar interactions can happen [53].

5. *S. aureus*

Staphylococci are spherical, non-sporulating, Gram-positive bacteria that are found in irregular grape-like clusters. The genus *Staphylococcus* is comprised of at least 45 species, four of which, *S. aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, and *Staphylococcus saprophyticus*, are considered the most important in clinical terms [54]. *S. aureus* is considered an opportunistic pathogenic bacterium, causing a considerable number of diseases ranging from superficial skin and soft tissue infections (SSTI) to invasive infections, sepsis, and death. So, in the United States, the mortality rate due to *S. aureus* sepsis has only been about 20,000 deaths per year in recent years [55]. Therefore, this bacterium has created a resistance to the best available antistaphylococcal agents, such as penicillin and methicillin. However, researchers have developed strategies to inhibit the quorum sensing control of *S. aureus*, since the toxins, virulence factors, and biofilm formation of this pathogen are controlled by the Agr (accessory gene regulator) quorum sensing system [55, 56].

5.1 Quorum sensing and related genes

The expression of the virulence factors of *S. aureus* is controlled by the Agr-QS system, which is responsible for causing genetic adaptations for intracellular communication. Likewise, the Agr of *S. aureus* is characterized mainly by regulating the expression of different toxins, virulence factors and controlling the interaction of bacteria-host at the infection site [57, 58]. Like many other bacterial physiological functions, the formation of *S. aureus* biofilms is mainly encoded by 12 different genes such as the fibrinogen-binding protein (Fib) gene, the fibronectin-binding protein (FnbA and FnbB) genes, intercellular adhesion genes (*ica*A, B, C, and D), clumping factor (*clf*A and B), elastin-binding protein (elastin-binding protein of *S. aureus* (EbpS)), laminin-binding protein (Eno), and collagen-binding adhesin protein (Cna) [59].

The genes encode different surface proteins that allow *S. aureus* to adhere to, penetrate into, and colonize the host. Ultimately, it leads to biofilm formation and virulence. In the *S. aureus* biofilm, the *fib* gene is responsible for facilitating and encoding the recognition of surface fibrinogen-binding proteins, while Cna promotes adhesion to the surface. For their part, the intracellular adhesion genes *ica*A, B, C, and D encode the process of cell-to-cell adhesion and start the formation of biofilms. While the clumping factor genes *clf*A and *clf*B encode cell wall-anchored proteins that bind to host surface fibrinogen [59, 60], facilitating *S. aureus* colonization, biofilm formation, and eliciting virulence via immune evasion through the binding of soluble fibrinogen.

5.2 Nanosystems tested *in vitro* and *in silico* for biofilm inhibition

Researchers have been seeking different strategies to counter *S. aureus* virulence factors, since virtually many of the toxins and other *S. aureus* virulence factors are controlled by the Agr quorum sensing system. For this reason, scientists have dedicated themselves to investigating strategies that manage to inhibit the QS control of *S. aureus* [61]. **Table 6** shows some studies of nanosystems synthesized from different precursors, which aim to inhibit the formation of *S. aureus* biofilms.

Nanosystem	Synthesis process	Chemical precursor	Antibiofilm activity assay %	Receptor protein (genes)	Reference
Graphitic carbon nitride nanosheets decorated with Cu-NPs	Cu-NPs: Cu precipitation and sonication. Carbon nitride nanosheets: melamine heating at 550°C for 4 h followed by precipitation.	$Cu(NO_3)_2$, SDS, L-ascorbic acid, and $C_3H_6N_6$	65% at 312.5 $\mu g \cdot mL^{-1}$	*ica*A	[62]
CS/Ag nanocomposite	Precipitation and heating at 90°C for 6 h	Ag^+ as precursor and CS as stabilizing reducing agent	96% at 250 $\mu g \cdot mL^{-1}$	†	[63, 64]
CS-ZnO-gentamicin nanocomposite	Precipitation and drying of NP at 60°C	$Zn(NO_3)_2$	77% at 0.125 $\mu g \cdot mL^{-1}$	†	[65]

†Not declared.

Table 6.
Biofilm formation inhibitors nanosystems tested in vitro *and* in silico *against* S. aureus*.*

Biofilm inhibition in the *S. aureus* isolate was shown to be independent of the *ica*A gene, leading to biofilm inhibition in *S. aureus* after treatment with Cu/g-C_3N_4 nanocomposites. On the other hand, Ramachandran [63] demonstrated that CS-loaded Ag-NPs favorably inhibited the formation of *S. aureus* biofilm at a concentration of 250 $\mu g \cdot ml^{-1}$. Therefore, they confirmed that there were damages in bacterial growth, arrest of survival, deformation of the membrane, and alterations of the exopolysaccharide when increasing the concentration of silver nanocomposites loaded with chitosan (CS). Finally, Hemmati [65] demonstrated that incorporating gentamicin-loaded ZnO-NPs into a chitosan solution developed a slow drug release rate compared to gentamicin-conjugated CS-ZnO NPs. Likewise, with the three components (gentamicin, chitosan, and ZnO), the scientists showed that the greatest antimicrobial and antibiofilm activity against *S. aureus* and *P. aeruginosa* occurred in the gentamicin-loaded CS-ZnO nanocomposite, due to the synergistic action that presented the gentamicin with the nanocomposite.

6. *L. monocytogenes*

Listeriosis is a serious infection that is usually caused by eating food contaminated with *Listeria*. In the United States, approximately 1600 people contract listeriosis each year, and approximately 260 people die from the disease [66]. Additionally, mortality from this infection can be as high as 30% in some parts of the United States. In European countries, the European Food Safety Authority (EFSA) reported a total of 1760 cases of listeriosis in humans in 2013 [67]. Contaminated ready-to-eat foods, such as soft cheeses made primarily from unpasteurized milk, smoked fish, ice cream, melon, apple, and vegetables, have been implicated in *L. monocytogenes* [68].

L. monocytogenes is a Gram-positive bacterium with a diameter of 0.5 to 4 μm and a length of 0.5 to 2 μm. It is a facultative anaerobic, catalase-positive, oxidase-negative,

non-spore-forming microorganism. It is generally motile due to the presence of flagella in a temperature range of 22–28°C, but immobile above 30°C. The growth temperature of *L. monocytogenes* is −0.4 to 45°C, with an optimum temperature of 37°C. The bacterium can survive in water activity <0.90 and pH 4.6–9.5 and tolerate salt levels (NaCl)) up to 20%. Furthermore, *L. monocytogenes* is resistant to disinfectants and can adhere to different surfaces [69, 70].

6.1 Quorum sensing and related genes

Biofilm formation of *L. monocytogenes* can be influenced by several external factors, such as growth and pressure conditions, temperature, growth method, physicochemical properties of the substrate, and the presence of other microorganisms [71], as well as internal factors such as *prf*A, *act*A, proteins encoded by the σ^B gene, and the ABC (ATP-binding cassette) permease transporter gene [72]. The *L. monocytogenes* genes involved in flagellar motility (*fli*Q, *fla*A, *fli*1, *mot*A) are required for biofilm formation, such as the PhoR gene (phosphate sensory operon) and the genes involved in D-alanine uptake in lipoteichoic cells [1].

L. monocytogenes is sensitive to a wide range of antibiotics active, except cephalosporins and fosfomycin, to which it has inherent resistance. The most common treatment for listeriosis is ampicillin or a combination of ampicillin with gentamicin; however, the *fos*X, *lin*, *abc-f,* and *tet*(*M*) genes are the four most common antimicrobial resistance genes found in *L. monocytogenes* in cases of foodborne transmission [73].

6.2 Nanosystems tested *in vitro* and *in silico* for biofilm inhibition

The reduction and elimination of biofilm of *L. monocytogenes* have been studied through the synthesis and application of nanoparticles and nanosystems. In addition, this nanotechnology can be green technology (**Table 7**).

Synthesized ZnO nanostructures from *Nigella sativa* seed affect biofilm without influencing the bacterial growth, resulting in the formation of weak biofilms possibly

Nanosystem	Synthesis process	Chemical precursor	Antibiofilm activity assay %	Receptor protein (genes)	Reference
Cu-NPs	Inert gas condensation	Pure copper	†	†	[74]
ZnO nanostructures from *Nigella sativa* seed	Microwaves	Zinc nitrate and *Nigella sativa* seed extract	91% (256 μg·mL^{-1})	†	[75]
Superparamagnetic IO-NPs	Precipitation and heating at 80°C for 1 h	Ferric chloride and ferric sulfate with polyethylene glycol	88% (16 μg·mL^{-1})	†	[76]
Ag-NPs from grown shoots of *Tamarix nilotica*	Bio-fabrication of Ag-NPs with *T. nilotica*	Silver nitrate with *T. nilotica* extract	62–64% (8 μg·mL^{-1})	†	[77]

†Not declared.

Table 7.
Biofilm formation inhibitor nanosystems tested in vitro *and* in silico *against* L. monocytogenes.

by reducing the surface adhesion and subsequent microcolony formation [75]. Although further research is necessary to unearth the plausible mechanism of biofilm inhibition by the ZnO nanoparticles.

In superparamagnetic iron oxide (IO) nanoparticles, biofilm reduction is attributed to the generation of reactive oxygen species (ROS) due to the interactions of the nanoparticles in the microorganism [76]. Similarly, as shown by Al-Shabib [77], green synthesis of silver nanoparticles produces ROS, causing cell death and inhibition of biofilms. Also, enhanced ROS production as the plausible mechanism of antibiofilm action is described [78] since gold nanoparticles interfere with the EPS matrix and disintegrate the architecture of the biofilm.

7. *Clostridium*

Clostridium is a genus of bacteria that include more than 100 species, is categorized as a Gram-positive bacterium, has flagella, and is anaerobic. They can cause multiple foodborne diseases in humans, such as botulism, *C. perfringens* food poisoning, necrotizing enteritis, and others [5]. These microorganisms can sporulate, which increases their resistance, and they can spread through abiotic surfaces.

In addition, some species can form biofilms, which are beneficial in some industrial processes (recycling and cellulose degradation processes, gas, acetone, butanol, and ethanol production) [5, 79, 80]. There are also some non-pathogenic bacteria in humans that form this type of biofilm and are part of the intestinal microbiota, such as *Clostridium clostridioforme* and *Clostridium malenominatum* [5].

In the case of pathogenic bacteria that cause diseases in humans, biofilms produced from bacteria can be a significant issue because this mechanism protects the microorganisms from antibiotics, the environment, toxic molecules, certain stress conditions, and immune system responses, hence, biofilm helps bacteria survive and may play a role in virulence [5, 6].

Specifically, the *Clostridium* species transmitted by food contamination that causes virulence in humans with the ability to form biofilms are *C difficile*, *C. botulinum,* and *C. perfringens* [5].

C difficile. *Clostridium difficile* infections (CDI) from *C. difficile* are the most prevalent cause of nosocomial diarrhea and colitis in the United States [81, 82]. This species develops in the colon after antibiotic medication changes the gut microbiota and secretes toxins that are virulence factors, including A and B toxins [80, 81, 83].

C. botulinum. These species produce one of the most lethal substances known to induce botulism, botulinum neurotoxin (BoNT). Botulinum toxin inhibits nerve function and can induce paralysis of the respiratory and muscular systems [84].

C. perfringens. One of the most common causes of foodborne infection in the United States. It can produce numerous toxins and is the cause of gas gangrene, necrotizing enteritis, food poisoning, and diarrhea associated with antibiotics [6].

7.1 Quorum sensing related genes and nanosystems' evaluation

Into the *Clostridium* genus, the implicated QS genes of *C. perfringens* are well established. QS is performed by the Agr and LuxS systems that are involved in toxin production and pathogenicity through propeptides and AI-2 production, respectively [85]. Although CDI have been widely reported and associated with *C. difficile* toxin production, the QS system by which these infections are regulated is considered a

complex multifactorial process [86]. There are even few reports on the effect of nanosystems involved in biofilm and QS inhibition in *Clostridium*.

In a study, Omoigberale, see [87], it has been found that Ag-NPs at a concentration of 10 mg·mL^{-1} can generate a biofilm reduction of 19–58%, while Au-NPs generate a smaller reduction with 12–39%, at the same concentration. However, this percentage range of reduction is considered strain-dependent, since this behavior was evaluated in 17 strains of *Clostridia*. At the same time, Au-NPs/Ag-NPs-Gentamicin complexes were evaluated obtaining 31% and 30% biofilm reduction, respectively. Although it was not a better performance than that obtained for the simple metallic nanoparticles, it did have a better effect compared to other antibiotics tested in the nanosystems.

Silver nanoparticles (Ag-NPs) also have been evaluated on specific isolates of *C. perfringens*, finding biofilm inhibition percentages of 80.8–82.8% at concentrations up to 100 μg·mL^{-1}, but showing the same behavior as in previous studies [87] in which these percentages are strain-dependent, or in this case, isolate-dependent from different animal and human organisms [88].

Undoubtedly, the molecular study of nanosystems with inhibitory QS potential is a great area of opportunity for this genus, pursuing to elucidate the signaling mechanisms and QQs that could counteract its pathogenicity.

8. Conclusions and perspectives

Biofilm formation is nowadays a worldwide topic of interest for public health due to the importance in pathogen survival. Knowing the mechanism of synthesis can help to develop new materials to prevent and eliminate bacteria. Quorum sensing is one of the mechanisms through which bacteria can communicate and create barriers against antibacterial agents. Nanosystems applied in food safety may interact with genes that express the signalization of quorum sensing, as many authors have reported. Understanding the interaction of the nanoparticle with the DNA of cells may lead to the formulation of new materials that exhibit this inhibition route, and for that, molecular docking can help to elucidate the possible interaction and, further, allow the food industry to be even safer for all populations.

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Conflict of interest

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