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AN OVERVIEW OF *SALMONELLA* BIOFILMS AND THEIR INHIBITION BY PLANT BIOACTIVE COMPOUNDS

Running title: *SALMONELLA* BIOFILMS AND INHIBITION BY BIOACTIVES

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Microbial biofilms are ubiquitous and highly successful forms of life. Among them, species found in food industries often include human pathogens like *Salmonella* spp., a significant concern due to its ability to adapt and survive in diverse environmental stresses. This bacterium is a major global public health issue, causing foodborne illnesses with considerable morbidity and economic costs. Combatting bacteria within biofilms necessitates novel strategies, and recent research highlights natural compounds as promising agents due to their antibiofilm, antivirulence, and antimicrobial properties. Specifically, plant-derived compounds have shown potential to modulate biofilm formation either alone or in combination with other substances. This review provides a comprehensive overview of *Salmonella* biofilms, encompassing their development, composition, resistance mechanisms, and tolerance mechanisms. It focuses particularly on the inhibition of these biofilms by plant-derived compounds.

Keywords: Antimicrobials. Bioactive molecules. Essential oils. Plant extracts. Quorum sensing.

INTRODUCTION

Biofilms are responsible for conducting different biogeochemical cycles of elements in water, soil, sediments, among others, and have been considered as one of the best distributed and successful life forms on the planet (Flemming *et al*., 2016). They can be found on different abiotic and biotic surfaces and biofilms are affected by several aspects, revealing their dynamic and sometimes unpredictable behaviors. The process of biofilm formation depends on factors such as cellular interactions, the surface where it adheres to, environmental conditions, besides the microbial species within it (Dass, Wang, 2022; Borges *et al*., 2018; Flemming *et al*., 2023).

Biofilms also cause the deterioration of the systems in which they are formed, leading to corrosion of pipes and equipment for example, making them a problem in different areas, including the food industry. Biofilms have been found as reservoirs of recurrent bacterial contamination, which can lead to food spoilage and foodborne illnesses (Brooks, Flint, 2008; Carrascosa *et al*., 2021). Many of the biofilm-forming species found in food factories are human pathogens, which can form biofilms on different surfaces common in food industries such as stainless steel, glass, wood, polypropylene, among others (Galié *et al*., 2018). These materials are very common in dairy and poultry industries, where various processes and structures such as milk tanks, pasteurizers, pipes, cutters, tables, employee gloves, and packing material act as surfaces for different colonizing species at various temperatures. Food matrices can be contaminated with bacterial cells detached from these biofilms, potentially affecting human health when these contaminants are foodborne pathogens (Carrascosa *et al*., 2021; Galié *et al*., 2018).

According to World Health Organization, every year 1 in 10 people fall ill and 33 million of healthy life years are lost due to foodborne diseases. These ailments can be severe and diarrheal diseases are the most common illnesses resulting from unsafe food, being *Salmonella* 1 of the 4 key global causes of diarrheal diseases (WHO, 2018). *Salmonella* is a genus of Gram-negative bacteria that represents one of the major public health problems worldwide, since its diverse group of serotypes mostly cause foodborne illnesses to humans, resulting in significant morbidity and economic impacts (Merino *et al*., 2019). The Center for Disease Control and Prevention (CDC) estimates that each year in the U.S., there are about 1.35 million cases of salmonellosis, with 26,500 hospitalizations and 420 deaths, being contaminated food the main source of the cases. Salmonellosis is the second leading cause of foodborne illnesses in the U.S., after norovirus, but is the leading cause of hospitalizations and deaths from food poisoning (FDA, 2023).

In addition to its clinical significance due to causing chronic infections, *Salmonella* is also of great concern in the food industry, as it can adapt and survive in many stressful environmental conditions. Approximately 50% of the strains isolated from poultry farms have the capacity to produce biofilms in processing areas such as walls, floors, pipes, and drains and in contact surfaces with materials like stainless steel, aluminum, nylon, rubber, plastic, polystyrene, and glass (Marin, Hernandiz, Lainez, 2009; Shatila, Yaşa, Yalçın, 2021).

Bacteria living in biofilms have higher tolerance to antibiotics and disinfectants compared to their planktonic state. Thus, novel strategies are needed to combat bacteria in biofilms (Lu *et al*., 2019; Rossi *et al*., 2022). This area of research has been extensively explored in recent years, and natural bioactive compounds are commonly reported to hinder biofilm formation alone or in association with other agents due to their antibiofilm, antivirulence, and antimicrobial properties. They comprise plant extracts (Alam *et al*., 2020), enriched extract fractions (Slobodníková *et al*., 2016; Zamuz *et al*., 2021), essential oils (Rossi *et al*., 2022), isolated compounds (Santos *et al*., 2021), among others.

This review aims to provide an overview of *Salmonella* biofilms, their development, matrix composition, resistance and tolerance to biocides and antimicrobials, and inhibition by plant-derived compounds.

MATERIAL AND METHODS

In this literature review, studies have been analyzed to elucidate key aspects of *Salmonella* biofilms, including their development, matrix composition, resistance mechanisms, and inhibition by plant-derived compounds. The goal was to provide an overview of biofilms, with emphasis in their ecological significance and their outstanding ability to persist in diverse environments.

An in-depth literature search was conducted using established databases such as Scopus, PubMed, Web of Science, SciELO, Google Scholar, and ScienceDirect, without imposing data restrictions. However, priority was given to recent studies, particularly those published within the last five years, to ensure that the review reflects the most current advancements in the field. This search culminated in the creation of Table 1, which compiles studies that report on the inhibition of *Salmonella* biofilms by the natural compounds. Key search terms utilized included: “biofilm”, “biofilm inhibition”, “*Salmonella*”, “quorum sensing inhibition”, “plant-derived compounds”, “phenolic compounds”, and “essential oils”, often combined using the AND operator to refine the results. The selection process involved a critical examination of article abstracts to ensure relevance to the scope of this review.

The study gathered data regarding the general composition of *Salmonella* biofilms, identifying the factors that contribute to their antimicrobial tolerance, and exploring the various inhibition mechanisms that have been investigated over the years. Then, the article delved into the specific inhibition of *Salmonella* biofilms by natural compounds, with a focus on essential oils, plant extracts, and isolated compounds. The review also aimed to assess whether natural compounds have been studied in sufficient detail, particularly concerning their potential mechanisms of action and the analytical methods employed to evaluate their efficacy against biofilms.

GENERAL STAGES OF BIOFILM DEVELOPMENT

Bacteria live in association and not simply as autonomous entities. Biofilms are the clearest example of this association as they are organized aggregates of microorganisms attached to an abiotic or biotic surface protected by extracellular polymeric substances (EPS) produced by themselves, forming channels that allow the passage of water, enzymes, nutrients, and residues (Flemming *et al*., 2023). The EPS is mainly composed of carbohydrates, proteins, lipids, and exogenous DNA (eDNA) (Flemming *et al*., 2016; Lu *et al*., 2019). The biofilm architecture has water channels for nutrient transportation in between the EPS matrix. The main role of this matrix is to facilitate the adherence of microbial cells to a surface and to each other, and additionally it provides protection for resident bacteria against a variety of environmental stresses such as adverse temperature, nutritional alterations, osmotic shock, drying, and ultraviolet (UV) radiation (Jamal *et al*., 2015; Kassinger, van Hoek, 2020).

Due to the properties of the matrix and the physical interactions between the biofilm cells, the lifestyle within a biofilm is completely different from those cells found in the free-living form, called planktonic cells. The extracellular polymeric substance (EPS) matrix enables bacterial cells within the biofilm to stay united and protected, thereby granting them greater resilience against environmental conditions and host defenses compared to planktonic cells (Carrascosa *et al*., 2021; Dass, Wang, 2022; Flemming *et al*., 2023).

The biofilm development is a transition process from the planktonic state to the sessile form, and these two life forms are genetically distinct. The genetic transition occurs throughout the biofilm life cycle and involves four distinct stages, as described below, and illustrated in **Figure 1**.

Stage 1. Adsorption - Reversible attachment

This process begins with the interaction between a few planktonic cells and the substrate and takes only a few seconds to initiate. In this step, a thin layer of organic and inorganic particles is formed. Basically, any substance present in the liquid medium that is transported by Brownian motion, sedimentation, or convection, becomes a conditioning layer that serves as the basis for biofilm growth (Jamal *et al*., 2015; Mahamuni-Badiger *et al*., 2020). At this stage, the chemotaxis process is important to direct planktonic cells to the source of nutrients and then promote microbial attachment and growth in the community. When the cells reach the surface, the interaction depends on the sum of attractive and repulsive forces between both surfaces (Muhammad *et al*., 2020). The planktonic cells adhere to the substrate by nonspecific physical forces, such as electrostatic forces, hydrophobic interactions, and Van Der Waals forces (Crouzet *et al*., 2014; Khatoon *et al*., 2018). In this reversible stage, the cells remain in Brownian motion and can be easily detached due to bacterial motility through appendages such as flagella, fimbriae, and pili, or due to the action of opposing forces. This process depends on aspects such as the amount of energy available, temperature, pressure, hydrophobic or hydrophilic characteristics of the contact surface, and whether the repulsive forces on the surface are greater than the attractive ones (Jamal *et al*., 2015; Khatoon *et al*., 2018; Muhammad *et al*., 2020).

Stage 2. Adhesion - Microcolony formation

The attachment of bacteria to the substrate at this stage is irreversible and depends on the bacterial physical appendages such as flagella, fimbriae, and pili, and how these manage to overcome the repulsive physical forces of the conditioning layer. When such bacterial appendages meet the conditioning layer, there is a stimulation of chemical reactions such as oxidation, which consolidates the bonding of the bacteria with the surface. At this stage, more bonding forces appear, such as dipole-dipole, hydrogen bonds, ionic and covalent bonds, and hydrophobic interactions, all of which are responsible for the so-called irreversible adhesion. The bacteria begin to multiply and release chemical signals that allow them to communicate through a process called quorum sensing (QS), which activates the genetic mechanisms needed to produce EPS and begin the biofilm maturation process (Jamal *et al*., 2015; Mahamuni-Badiger *et al*., 2020; Solano, Echeverz, Lasa, 2014).

Stage 3. Biofilm maturation

The bacteria adhered to the surface continue to divide by binary fission and remain aggregated in the form of micro-colonies, decreasing motility. The production of signaling molecules is also continuous at this stage, which keeps the genetic systems for EPS production activated. The growth of the micro-colonies and the EPS matrix allow the development of a three-dimensional structure, normally referred to as a mushroom-like structure, that contains water-filled channels to transport nutrients within the inner parts of the biofilm and remove waste materials, as well as providing greater protection against antimicrobials and host defense mechanisms (Jamal *et al*., 2015; Muhammad *et al*., 2020).

Stage 4. Dispersion of bacteria

The dispersal process can occur in response to changes in the microenvironment such as oxygen availability, access to nutrients, and changes in temperature. These factors that were initially important for bacterial survival and optimization of available resources become signals that activate dispersion. The bacteria in the innermost parts of the biofilm face a stressful condition generated by hypoxia, the scarcity of signaling molecules, and the low growth rate (Guzman-Soto *et al*., 2021). This causes the activation of regulatory mechanisms, remodeling the biofilm structure by the production and release of enzymes that degrade the EPS matrix components, by interruption of non-covalent interactions, and cell death, which produces cavities within the biofilm (Solano, Echeverz, Lasa, 2014). These cavities serve as escape or dispersal routes, releasing cells individually or in groups, leaving them free to spread out and colonize new substrates (Guzman-Soto *et al*., 2021).

Researchers are now focusing on inhibiting biofilm formation through various strategies that target the different stages of the process of biofilm formation. These approaches involve preventing cell receptors from recognizing surfaces, inhibiting bacterial adhesion, or identifying compounds that can eliminate formed biofilms (Figure 1).

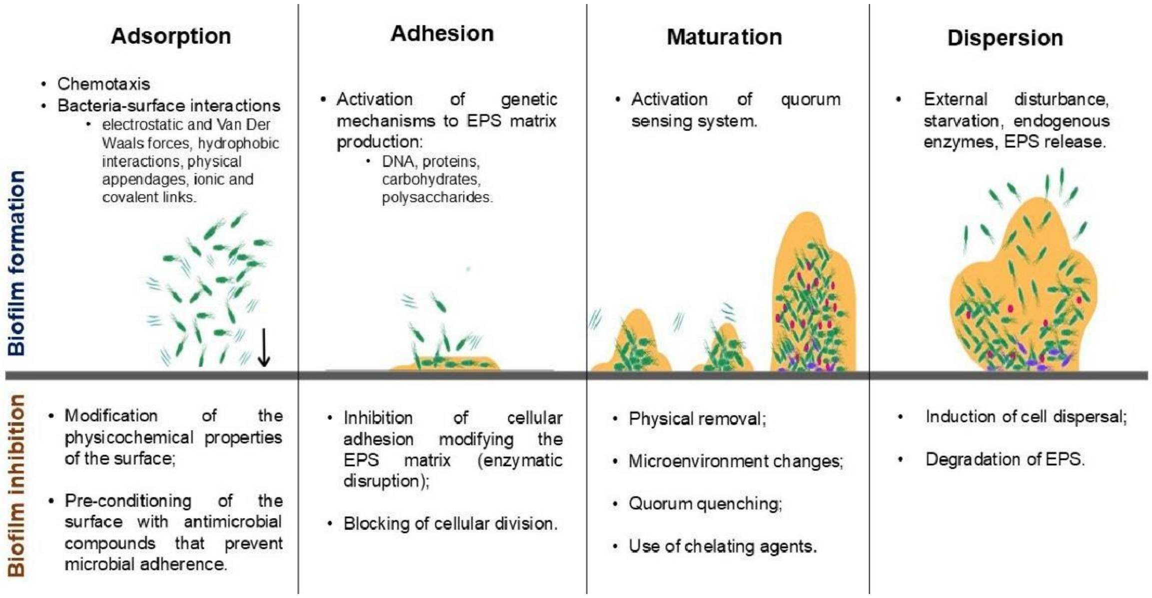

FIGURE 1 - Stages and physicochemical processes involved in biofilm formation and different inhibition strategies in each of the stages.

MATRIX COMPONENTS OF *Salmonella* BIOFILMS

The composition of the bacterial biofilm matrix is complex and can vary greatly between species and even within the same species under different environmental conditions. Its primary component is water, which is responsible for the flow of oxygen and nutrients within the biofilm. The main structural and functional elements of the matrix are carbohydrates, proteins, lipids, lipopolysaccharide (LPS) and eDNA (Flemming *et al*., 2023; Jamal *et al*., 2015). *Salmonella* and other members of the *Enterobacteriaceae* family produce an extracellular matrix composed mainly of curli fimbriae, extracellular cellulose, and biofilm-associated protein (BapA), in response to environmental stress (Sakarikou *et al*., 2020).

Curli fimbriae is an amyloid-like cell-surface fiber that acts as a promoter of community behavior through the formation of biofilms, being involved in the processes of surface adhesion and cell aggregation (Jonas *et al*., 2007; Keelara, Thakur, Patel, 2016; Solomon *et al*., 2005). It is the protein with the highest content in the biofilm matrix produced by many Enterobacteriaceae including *Salmonella* spp. and *Escherichia coli* (Smith *et al*., 2017). In *Salmonella* spp., the curli fimbriae is encoded by two divergently transcribed operons *csgDEFG* and *csgBAC* (*agfDEFG* and *agfBAC* depending on the annotation, respectively). The *csgBAC* operon encodes the structural subunits of curli, CsgB and CsgA, as well as the chaperone CsgC that prevents these subunits from forming an amyloid structure before being transported to the cell envelope. The *csgDEFG* operon is responsible for encoding the accessory proteins required for curli assembly. CsgE, CsgF, and CsgG participate in translocation and assembly of the structural subunits of curli into the cell envelope. CsgD is known as a master regulator of biofilm development and stimulates the production of curli by transcriptional activation of the *csgBAC* operon (Evans *et al*., 2015; Bhoite *et al*., 2019; Jonas *et al*., 2007; Keelara, Thakur, Patel, 2016; Solomon *et al*., 2005).

The expression of a proteinaceous component of the *Salmonella* extracellular matrix, BapA, is synchronized with the expression of curli and extracellular cellulose through the action of the regulatory protein CsgD (Jonas *et al*., 2007). BapA plays an important role in bacterial aggregation and biofilm formation at the air-liquid interface through homophilic interactions between bacterial cells (Lamas *et al*., 2021). This protein is secreted through a type I secretion system, BapBCD, all encoded by the same operon, and have been studied specially in *Salmonella* Enteritidis (Latasa *et al*., 2005).

Another matrix-component commonly found in *Salmonella* biofilms is extracellular cellulose, a polysaccharide composed of D-glucose units linked together by *β*-1-4 glycosidic bonds. Cellulose biosynthesis occurs by expression of the *bcsABZD* and *bcsEFG* operons and is also upregulated by the master biofilm regulator CsgD, which stimulates transcription of the diguanylate cyclase AdrA at the post-transcriptional level, important for activating cellulose production (Jonas *et al*., 2007; Peng, 2016). BcsA and BcsB form the two subunits of the cellulose synthesis complex located in the cell envelope and they are responsible for the conversion of UDP-glucose to cellulose. The other proteins synthesized by the two *bcs* operons are involved in the regulation of cellulose synthesis and the cellular localization of the synthesis complex (Römling, Galperin, 2015). Higher cellulose production has been shown to be associated with increased thermal and chemical resistance of biofilms and it occurs at temperatures ranging from 15 to 25 °C, which are very relevant temperatures in the food industry (Kim, Jyung, Kang, 2022).

The presence or absence of cellulose and/or curli fimbriae determines the four morphotypes that can be observed in *Salmonella* colonies on Congo red agar plates: strains expressing cellulose and curli appear as red, dry, and rough colonies (RDAR); those expressing cellulose but not curli show the pink, dry, and rough morphotype (PDAR); if only curli is expressed, the colonies show a brown, dry, and rough morphotype (BDAR); when colonies appear smooth and white (SAW) it is possible that the transcriptional regulator CsgD is not active in this strain (Römling, 2005; Jain, Chen, 2007; Ćwiek, Bugla-Płoskońska, Wieliczko, 2019; Nesse *et al*., 2021). The detection of these morphotypes can indicate the degree of resistance and survival of *Salmonella* biofilms to different conditions, since cellulose plays a key role in increasing resistance to heat and chemical compounds (Solano *et al*., 2002; Villa-Rojas *et al*., 2017; Kim, Jyung, Kang, 2022).

The presence of different fatty acids and LPS was also noticed in the EPS fraction of RDAR expressing *S.* Enteritidis strain (Gibson *et al*., 2006). The LPS also improves surface wettability, required for swarm colony expansion, and LPS mutations may be able to induce alternative pathways leading to extracellular matrix production (Steenakers *et al*., 2012).

INFLUENCE OF SIGNALING MOLECULES ON BIOFILM FORMATION

The cell-cell communication by QS is used by bacteria to collectively adapt by activating or repressing genes involved in collective group behavior (Bassler, 2002; Lima, Winans, Pinto, 2023). This mechanism occurs through signaling molecules, also known as autoinducers (AI), which accumulate in the medium as a function of population density and can regulate the expression of genes (Fuqua, Winans, Greenberg, 1994; Bassler, 2002; Lima, Winans, Pinto, 2023). Many cellular processes are modulated by QS including sporulation, synthesis of antimicrobial peptides, regulation of virulence factors, and biofilm formation (Whitehead *et al*., 2001; Papenfort, Bassler, 2016; Lima, Winans, Pinto, 2023).

The connection between QS and biofilm formation has been widely described. QS is an important factor in the transition from simple aggregates of microorganisms in the early stages of the biofilm formation to its complex mature structure. Many bacterial species use QS to coordinate some steps in the biofilm formation process like microcolony formation, maturation, and dispersion, and some factors such as biofilm structure, nutrient acquisition, and regulation of antimicrobial resistance (Solano, Echeverz, Lasa, 2014; Guzmán-Soto *et al*., 2021; Khalid *et al*., 2022).

In *Salmonella* spp., different QS systems are known to act in the regulation of virulence factors, including biofilm formation. Communication involving autoinducer type 1 (AI-1) is incomplete for this bacterium, as it does not contain a homologue of the *luxI* gene that codes for the AHL synthase. However, despite not producing its own signaling molecule, *Salmonella* spp. express a transcriptional regulator homologous to LuxR, known as SdiA, which detects AHLs produced by other bacterial species (Michael *et al*., 2001; Dyszel *et al*., 2010; Smith, Ahmer, 2003; Smith *et al*., 2008; Almeida *et al*., 2017; Sholpan *et al*., 2021). Campos-Galvão *et al*. (2016) showed that the addition of a mixture of AHLs, with carbon chains ranging from 6 to 12 carbons, did not interfere with growth, but promoted the biofilm formation by *Salmonella enterica* serovar Enteritidis PT4 578 on polystyrene coupons under anaerobic conditions. Nonetheless, the addition of N-dodecanoyl-homoserine lactone (C12-HSL) induced a denser and more developed biofilm, optimized metabolism, enhanced tolerance to acidic stress and cationic peptides, while also upregulating the expression of virulence-associated genes *hilA*, *invA*, and *invF*, as well as genes linked to biofilm formation, namely *glgC*, *fliF*, *lpfA*, and *fimF* (Campos-Galvão *et al*., 2016; Almeida *et al*., 2017; Carneiro *et al*., 2020; Freitas *et al*., 2020). On the other hand, C12-HSL did not influence biofilm formation by *S.* Enteritidis PT4 578 at 28 °C under both aerobic and anaerobic conditions. However, at 37 °C, biofilm formation was reduced under aerobic conditions and increased under anaerobic conditions. Furthermore, under anaerobic conditions at 37°C, the expression of the *adrA* and *luxS* genes increased, suggesting an increase in c-di-GMP levels, a second messenger that controls essential physiological functions in bacteria (Carneiro *et al*., 2024). Molecular docking analysis confirmed the stronger binding affinity of C12-AHL with the SdiA protein, which may account for the varying levels of regulation by different AHLs (Almeida, Pinto, Vanetti, 2016). Addition of N-butyryl-homoserine lactone (C4-HSL) and N-hexanoyl-homoserine lactone (C6-HSL) also enhanced biofilm formation in *S.* Typhimurium cultivated aerobically (Bai, Rai, 2016). However, the biofilm formation of *S.* Typhimurium was not influenced under aerobic conditions by cell-free supernatant (CFS) from *Hafnia alvei* containing AHLs or by the addition of N-3-oxo-hexanoyl-homoserine lactone (3-oxo-C6-HSL) (Blana, Georgomanou, Giaouris, 2017). It is noteworthy that CFS contained metabolites other than AHLs that may have interfered with cellular responses to these AI (Vanetti *et al*., 2020). The AHL, C6-HSL and *N*-octanoyl-homoserine lactone (C8-

HSL) increased the invasion of HEp-2 cells by *Salmonella enterica* serovar Typhi (Nesse *et al*., 2011) and C8-HSL increased adhesion of this bacterium to HeLa cells (Liu *et al*., 2014).

Salmonella spp. also uses the AI-2 signaling molecule, synthesized by the LuxS protein, targeting the *lsrACDBFG* operon, which captures and processes AI-2 itself (Cox, McClelland, Teplitski 2013). It has been shown that the LuxS/AI-2 QS system is required for resistance to bile during biofilm formation by *S.* Typhimurium (Tsai *et al*., 2020).

The AI-3 is an aromatic amino compound belonging to the pyrazinone family produced by bacteria from the intestinal microbiota and by some enteric pathogens such as *Salmonella* and *E. coli* (Rul, Monnet, 2015). This AI along with epinephrine and norepinephrine secreted by eukaryotic cells are recognized by the same receptor, the sensor kinase QseC. Upon detecting these molecules, QseC phosphorylates the QseB response regulator, which in turn activates its own expression and the expression of target genes. Additionally, QseC also activates the transcription of the two-component QseEF system, which may also play a role in detecting host hormones (Sperandio *et al*. 2003; Hughes, Sperandio, 2008; Hughes *et al*., 2009). Hiller *et al*. (2019) evaluated biofilm formation by *S.* Enteritidis isolates in the presence of different concentrations of epinephrine and norepinephrine at 12 and 25 °C. They demonstrated a greater number of biofilm-producing isolates at 25 °C than at 12 °C, regardless of treatment. Furthermore, biofilm formation was not influenced by the presence of hormones, except for norepinephrine at 100 µM, which stimulated biofilm formation at 12 °C (Hiller *et al*., 2019).

Another important regulation system in biofilm formation is cyclic diguanosine monophosphate (c-di-GMP) signaling pathway. The c-di-GMP is an important second messenger molecule that exists widely in bacteria and is a central regulator of *Salmonella* biofilm formation (Tsai *et al*., 2020; Miller *et al*., 2022). In brief, CsgD is a key regulator of the *csg* gene cluster and regulates many genes involved in biofilm formation. CsgD expression is regulated by environmental stimuli and the levels of c-di-GMP. High intracellular levels of c-di-GMP promote CsgD activation, leading to the activation of genes involved in the production of curli and expression of AdrA, which increases cellulose biosynthesis. Thus, c-di-GMP stimulates the production of matrix components such as cellulose and curli, promoting biofilm formation, and inhibits motility (Miller *et al*., 2022; Ryan *et al*., 2006). In *S.* Typhimurium, increased c-di-GMP levels lead to increased cellulose synthesis and biofilm formation (Tsai *et al*., 2020). c-di-GMP reversely regulates biofilm formation and motility in *S.* Typhimurium, contributing directly or indirectly to the regulation of the RDAR morphotype, a biofilm phenotype characterized by the expression of cellulose and curli fimbriae (Ahmad *et al*., 2011).

RESISTANCE AND TOLERANCE OF *Salmonella* BIOFILMS

Biofilms are renowned for their enhanced ability to withstand various stress conditions compared to planktonic cells (Soto, 2013; Flemming *et al*., 2023). When faced with starvation, oxygen deprivation, and restricted metabolic flow, bacteria within biofilms can initiate a stringent response that triggers the activation of the SOS system, thereby promoting their survival (Drescher *et al*., 2019). Additionally, biofilms exhibit a tolerance to antibiotics that is 100 to 1000 times greater than that of free-living cells, which makes treating bacterial infections involving biofilms more challenging. Although some antimicrobial agents may reduce bacterial counts within biofilms, they are rarely effective in completely eradicating the pathogens, which can result in recurrent infections (Dufour, Leung, Lévesque, 2010). Consequently, bacterial resistance to antimicrobial agents, including *Salmonella*, has emerged as a significant global concern (Ćwiek, Bugla-Płoskońska, Wieliczko, 2019).

Olson *et al*. (2002) reported that *Salmonella* spp. in the planktonic form was sensitive to the antibiotics enrofloxacin, gentamicin, ampicillin, oxytetracycline, and trimethoprim, while bacteria in biofilm form were sensitive only to enrofloxacin. Papavasileiou *et al*. (2010) investigated the susceptibility to antibiotics ampicillin, cefuroxime, cefotaxime, gentamicin, imipenem, cotrimoxazole, ciprofloxacin, and moxifloxacin of 194 *Salmonella enterica* strains isolated from children with gastroenteritis. Of these, 109 strains formed biofilms, and as expected, they demonstrated that the biofilm form showed increased antimicrobial resistance compared to the planktonic cells.

The resistance of *Salmonella* biofilms against a wide variety of biocides has also been reported. Tabak *et al*. (2009) evaluated the susceptibility of *S.* Typhimurium in both planktonic and biofilm form to triclosan, a biocide included in a wide variety of antiseptic products such as toothpastes, deodorants, soaps, and lotions. The study showed that triclosan at 600 to 2000 ppm was not effective in eradicating this microorganism in biofilm form due to reduced diffusion of the compound caused by the presence of the EPS.

Haubert *et al*. (2019) showed that 26 isolates of *Salmonella* were tolerant to benzalkonium chloride (BC) disinfectant, in addition to forming biofilms and presenting resistance to streptomycin, sulfonamides and tetracycline, which are antimicrobials commonly used in animal production.

Antimicrobial tolerance depends on the species, but there are several factors that can contribute to its increase in biofilms, such as the presence of the EPS matrix that limits antimicrobials transport; physiological changes in the bacterial cell due to low multiplication rate, lack of nutrients, and environmental stress; expression of heavy metal resistance genes; QS; the emergence of persister cells that have a broad tolerance to bactericidal agents; and the overexpression of efflux pumps (Mah, O’Toole, 2001; Merritt, Kadouri, O’Toole, 2005; Singh *et al*., 2017; Ćwiek, Bugla-Płoskońska, Wieliczko, 2019). Some of these factors are detailed below.

Slow penetration of the antimicrobial due to the exopolysaccharide matrix

Numerous studies have investigated the diffusion of antimicrobial agents through biofilms and have demonstrated that the matrix can serve as a selective barrier that restricts their penetration (Lewis, 2001; Singh *et al*., 2017; Shree et al., 2023). Some studies have shown that microbial tolerance exists due to a delayed penetration process. When antimicrobial treatment is performed, the biofilm cells at the top of the liquid interface die due a more direct exposure, while bacteria that are in the innermost layers of the biofilm generally survive (Lewis, 2001; Dufour, Leung, Lévesque, 2010).

The matrix can act as an active chemical barrier by binding to and sequestering positively charged antibiotics such as aminoglycosides, toxic heavy metals, and cationic antimicrobial peptides. However, for uncharged antibiotics such as β-lactams, this binding to the matrix is unlikely to occur, which provides little or no barrier to penetration (Dufour, Leung, Lévesque, 2010).

Tabak *et al*. (2009) demonstrated the effect of triclosan on *Salmonella* planktonic cells and biofilms. The pathogen's tolerance to the biocide in the biofilm was attributed to its low diffusion through the extracellular matrix and gene expression responses that provided increased tolerance to antimicrobials. Within the biofilm, triclosan positively regulated the *bcsA* and *bcsE* genes involved in cellulose synthesis, which caused *Salmonella* to intensify the exopolysaccharide production (Tabak *et al*., 2009).

Altered microenvironment and stress response

The environmental conditions within biofilms are not homogeneous throughout the structure. This causes the bacteria in different parts of the biofilm to have distinct physiological and metabolic characteristics (Crabbé *et al*., 2019). The cells on the surface of the biofilm resemble planktonic cells, while those in the deeper parts, where nutrients and oxygen are limited, have a lower growth rate. This directly influences tolerance to antimicrobials that act on cellular processes involved in bacterial multiplication, such as cell wall synthesis and DNA replication (Dufour, Leung, Lévesque, 2010).

Bacteria can express a coordinated stress response to switch to more tolerant phenotypes when presented with adverse environmental conditions such as starvation, heat or cold shock, cell density, pH, osmolarity, among others (Dufour, Leung, Lévesque, 2010; Banerji *et al*., 2022). In biofilms, bacteria encounter different microenvironments that can trigger this response. For example, the lack of an important substrate or the accumulation of harmful products can cause some bacteria to enter a non-growth state, which acts as a protective mechanism against death. In addition, changes in the pH or osmotic environment within the biofilm can lead to the induction of a stress response that can result in antibiotic resistance due to reduced porin expression in the cell envelope (Stewart, Costerton, 2001; Rode, Singh, Drescher, 2020).

Paytubi *et al*. (2017) investigated the impact of culture medium composition on biofilm formation in *Salmonella* and found that in nutrient-rich growth media like colonization factor antigen (CFA) medium, biofilms were predominantly formed at the air-liquid interface. However, in minimal medium, biofilms formed at the solid-liquid interface. The authors inferred that nutrient deprivation induced transcriptional expression of *csgD*, resulting in elevated expression of curli and cellulose. This suggests that nutrient availability is a key determinant for the spatial distribution of biofilms.

Persister cells

The formation of persister cells is another mechanism that contributes to antimicrobial tolerance. These specialized cells enter in an inactive state in response to environmental stress that activates toxin-antitoxin systems, allowing them to survive stressful conditions (Jayaraman, 2008; Wang, Wood, 2011). Persister cells constitute a small fraction of the bacterial population in the stationary phase of planktonic cells (up to 1% of the population) and in biofilms of different species and can resume their multiplication when they encounter favorable conditions (Dufour, Leung, Lévesque, 2010; Mah, 2012). These persister cells are phenotypically different from multiplying cells, which make them extremely tolerant to high concentrations of antimicrobials, mainly because most classes of antibiotics in use act on growing cells (Lewis, 2007). Importantly, these cells withstand antimicrobials because they are in an inactive state and not because they possess genetic alterations associated with resistance (Singh *et al*., 2009).

In *Salmonella*, this phenomenon was well demonstrated by Drescher *et al*. (2019), who tested four *S. enterica* serovars (Schwarzengrund, Agona, Infantis, and Enteritidis) that were able to generate persister cells after exposure to high concentrations of ciprofloxacin and ceftazidime. The persistence levels of these *S. enterica* serovars varied under different culture conditions, being higher in biofilms when compared to planktonic cells.

Efflux pumps

One mechanism that has also been associated with biofilm resistance to antimicrobials is the overexpression of efflux pumps. Efflux pumps are membrane bound protein structures capable of expelling toxic substances to the external environment, among them antimicrobials. They can be specific for a single antimicrobial agent or be considered multi-drug pumps, eliminating different classes of antimicrobials. Worryingly, their expression can be induced by exposure to sublethal concentrations of antibiotics (Khatoon *et al*., 2018).

A study conducted by Baugh *et al*. (2014) aimed to determine if the inhibition of multi-drug efflux pumps could prevent biofilm formation. Using *S.* Typhimurium mutants lacking different components of the AcrAB-ToIC system, these authors demonstrated that mutants in ToIC and AcrB, but not AcrA, exhibited transcriptional repression of genes involved in biofilm formation, such as those encoding curli, compromising their ability to form biofilms. They concluded that genetic and chemical inactivation of efflux pumps may be a promising antibiofilm strategy.

Salmonella BIOFILMS INHIBITION BY PLANT-DERIVED COMPOUNDS

The biofilm formation process involves different stages that can be targeted by several natural antibiofilm compounds, as illustrated in **Figure 1**. The initial stage is the most critical for biofilm development due to the need to synthesize structures that allow bacterial adhesion to the surface, such as fimbriae or pilus, which makes this stage a strategic point of inhibition. Another key factor in the biofilm formation process is QS, which has greatly increased academic interest in searching for new products that may interfere with this communication (Ta, Arnason, 2015; Deryabin *et al*., 2019; Sakarikou *et al*., 2020; Lima *et al*., 2023).

Plant extracts and phytochemicals have been widely used as antimicrobial agents due to their cost-effectiveness, great structural diversity, reduced possibility of resistance development because they act at different targets, and their eco-friendly characteristics (Oulahal, Degraeve, 2022; Sakarikou *et al.*, 2020). The plant secondary metabolites exhibit great chemical diversity and important properties such as antimicrobial activity, whether evaluated alone or in combination with other antimicrobials at appropriate concentrations. The groups of phytochemicals with known antimicrobial activity are generally phenolic compounds, terpenoids, essential oils, alkaloids, polypeptides, among others (Deryabin *et al*., 2019; Albuquerque *et al*., 2021). Here we show the state of the art on the use of essential oils (EOs), plant extracts and plant-derived compounds to inhibit *Salmonella* biofilm formation (Table I).

Essential oils (EOs)

The medicinal properties and antimicrobial activity of many plants are due in part to the presence of a high content of antimicrobial compounds in essential oils (EOs) including thymol, carvacrol, eugenol, menthol, limonene, among others. In many cases, the activity of these substances occurs at sub-minimum inhibitory concentration (sub-MIC) (Luís *et al*., 2017; Snoussi *et al.*, 2018; Pelarti *et al*., 2021; Rossi *et al*., 2022; Sateriale *et al.*, 2023). In addition, they also have the ability to affect cell wall integrity allowing the inactivation of bacterial cells without selecting for resistance, probably as a result of their multiple cellular targets. These compounds can also prevent bacterial adhesion and inhibit cell coaggregation, and can be widely used for their antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Maurya *et al*., 2021; Pelarti *et al*., 2021; Verešová *et al*., 2024) thanks to their easy availability, low toxicity and rapid degradation in the environment, making them safe agents with broad activity (Valeriano *et al*., 2012; Hakimi Alni, Ghorban, Dadmanesh, 2020; Rossi *et al*., 2022).

Several studies have demonstrated the effect of EO on *Salmonella* biofilm, summarized in Table I. Valeriano *et al*. (2012) evaluated the effect of a disinfectant solution formulated with the EO of peppermint (*Mentha piperita*) and lemongrass (*Cymbopogon citratus*) against biofilm formation by *Salmonella* Enteritidis S64 on stainless steel surfaces. After 10 min of exposure, a significant reduction in the bacterial populations of the formed biofilm was observed, and a 20 min treatment was sufficient to completely inhibit these bacterial populations. The authors assumed that this result may be due to increased permeability of the cytoplasmic membrane linked to the action of EO that cause a loss of important intracellular contents such as essential ions and molecules that can lead to cell death (Valeriano *et al*., 2012).

Amaral *et al*. (2015) evaluated the effect of carvacrol and thymol, the major components of the EO of oregano and thyme on biofilm formation of different *Salmonella* spp. strains on polypropylene. The microorganisms evaluated were *S.* Typhimurium ATCC 14028 and three strains isolated from food related to foodborne outbreaks: *S.* Enteritidis, *S.* Typhimurium and *S.* Saint Paul. The cells were quantified during and after biofilm formation in the presence of the evaluated compounds. The results showed that during biofilm formation, the two compounds at sub-MICs were able to reduce the content of planktonic cells between 1-2 logs CFU/mL, while the biofilm formed was reduced between 1-5 logs CFU/cm². The greatest reduction of biofilms occurred with carvacrol (5 log CFU/cm²) in *S.* Typhimurium ATCC 14028. Thymol showed the best reduction for *S.* Enteritidis, approximately 4 log CFU/cm². These results were confirmed with scanning electron microscopy (SEM) analyses, which showed a break in the structure of the biofilm and a diffuse adherence of the bacterial cells (Amaral *et al*., 2015). More recently, Sateriale *et al*. (2023) also observed the inhibition of *S*. Typhimurium biofilms by thyme EO, suggesting the use of this natural agent as a promising food preservative to counteract biofilm-related contamination in the food industry.

A similar study by Miladi *et al*. (2017) evaluated the bacterial susceptibility and biofilm eradication by carvacrol, thymol and eugenol, alone and in combination with nalidixic acid, against 12 strains of *S.* Typhimurium. The MIC and the minimum biofilm eradication concentration (BEC50) of the evaluated compounds and their combinations were determined. The biofilms were visualized by SEM on stainless steel surfaces after being exposed to the compounds. For most of the evaluated strains, it was observed that the BEC50 were higher than the concentration required to inhibit the multiplication of cells in suspension. All compounds showed synergism with nalidixic acid with a significant reduction in BEC50. SEM analyses showed that with a concentration of 8 µg/mL of nalidixic acid, the bacterial biofilm was reduced. However, when this compound was combined with ½ MIC of each of the compounds, the antibiofilm activity was greater than separately, which could mean that the essential oils could facilitate the entry of antibiotics into the biofilms formed by *S.* Typhimurium.

A recent study showed the effect of *Allium sativum* and *Cuminum cimynum* EO against planktonic growth, biofilm formation, and QS of strong biofilm-forming *S.* Typhimurium strains. The results showed that with a ½ MIC of both EO, the expression of QS genes (*sdiA* and *luxS*) and cellulose biosynthesis genes (*csgD* and *adrA*) were significantly reduced, in addition to a reduction in the amount of biofilm formed. Among the main components found in these essential oils are several sulfur compounds and pinene, carene, *α*- and *β*-terpineol, which are known to have antimicrobial and antibiofilm activity, interfering with motility as a result of reduced production of EPS (Hakimi Alni, Ghorban, Dadmanesh, 2020).

The EO of clove was tested by Somrani *et al*. (2022) and Alibi *et al*. (2022) against *S.* Enteritidis, achieving inhibitions of 50 and 98% at different concentrations and time of incubation. Alibi *et al*. (2022) also observed high biofilm inhibitions by EO of cinnamon (99%), thyme (96%) and rosemary (80%), suggesting that sanitizers based on essential oils could be a potential strategy to control biofilms in food-related environments.

Guillín *et al*. (2021) evaluated fifteen EO from medicinal aromatic plants for their anti-biofilm activity against *S*. Enteritidis ATCC 13076 and *S*. Typhimurium ATCC 14028.

Six EO showed anti-biofilm activity, and EO from *Lippia origanoides* chemotype thymol-carvacrol II (LTC II) presented the lowest MIC, MBC and the highest percentage of biofilm inhibition (>65%) on both microorganisms, which was confirmed by scanning electron microscopy (SEM) images. Transcriptional analysis showed that EOs could inhibit the expression of QS-related genes *(luxS, qseB, sdiA*) and biofilm formation genes (*csgA, csgB, csgD, flhD, fliZ,* and *motB*), indicating their potential use as anti-biofilm antimicrobial agents.

Selim *et al*. (2022) investigated the antimicrobial and antibiofilm effects of EO obtained from *Salvia officinalis* L. leaves from Saudi Arabia, against many *S. enterica* isolated from raw milk. The impact of EO on *Salmonella* biofilm development was qualitatively assessed, and EO at 5% showed an anti-biofilm activity on different isolates. According GC–MS analysis, the most prominent compounds on EO were 1,8-cineole (39.18%), β-caryophyllene (12.8%), and α-terpineol (10.3%). According to the authors, this was the first report for *S. officinalis* EO antibiofilm properties against *Salmonella*, and the EO may be used in the future for the development of antibacterial drugs.

Abdullah *et al*. (2021) investigated the chemical profile, antimicrobial, and mutagenic activities of the *Elletaria cardamomum* EO. The major bioactive components found by GC-MS were α-terpinyl acetate (35%), 1,8-cineole (25%), linalool acetate (8%) and sabinene (5%). Green cardamom EO at 0.015, 0.031, 0.062, and 0.125% (v/v) inhibited 6, 45, 50, and 100% of the *S.* Typhimurium JSG 1748 biofilm. The authors suggest that EO are safe organic antimicrobials and could possibly be used in the food industry as antimicrobials while at the same time imparting a pleasant and appealing aroma for consumers.

Pelarti *et al*. (2021) evaluated the effect of *Artemisia dracunculus* EO on *S.* Typhimurium biofilm formation. The major compound detected by GC–MS was estragole (64.94%). The MBC was 5 μl/ml and an inhibitory and disruption effect on the biofilm was observed at sub-MIC. Besides, significant downregulation of biofilm and QS-related genes (*luxS, pfs,* and *hld*) by treatment with MIC/2 was observed. Anti-biofilm, anti-QS, and non-toxicity of *A. dracunculus* EO was reported for the first time, encouraging their use as antimicrobial in many sectors.

Recently, Somrani *et al.* (2024) investigated the antibiofilm effects of essential oils (EOs) from cinnamon, garlic, and onion on *Salmonella* Enteritidis. Their study assessed the impact of these EOs on initial cell adhesion as well as the eradication of preformed biofilms. A dose-dependent effect was observed, with higher EO concentrations exhibiting greater efficacy against preformed biofilms. The EOs effectively inhibited bacterial adhesion and, to a lesser extent, facilitated biofilm removal.

Plant extracts

Plant extracts and plant-derived compounds have been extensively studied against *Salmonella* biofilm formation, as can be seen in Table 1.

Lou *et al*. (2016) observed a significant inhibitory activity of burdock (*Arctium lappa* L.) leaves extracts against the growth and biofilm development of *E. coli* and *S.* Typhimurium. At a concentration of 2.0 mg/mL, the inhibition reached 78.7 and 69.9%, respectively. The study suggested that phenolic acids contained in burdock leaf fraction, such as chlorogenic acid, rutin, quercitrin, luteolin, *p*-coumaric acid, caffeic acid, and quercetin, could help control foodborne pathogens and inhibit lipid oxidation, making this product effective against microbial growth and oxidative reactions in meat preservation (Lou *et al*., 2016).

A study conducted by Wu *et al*. (2016) determined the effect of *Ginkgo biloba* extract against biofilm formation of *Salmonella* spp. and *Listeria* spp. isolates from poultry. They observed that the antibiofilm activity was concentration dependent; showing that for *S.* Enteritidis, the *G. biloba* extract at 100 μg/mL had an inhibitory activity. The extract also reduced swarming motility of *S.* Enteritidis but induced swimming motility, which suggests that swarming motility influenced the biofilm formation of this bacterium. Flavonoids myricetin and quercetin, and terpenoids ginkgolides and bilobalides were the main functional compounds in the extract (Wu *et al*., 2016).

Olawuwo *et al*. (2022) determined the antimicrobial and antibiofilm potential of organic and aqueous extracts of leaves of *Alchornea laxiflora, Ficus exasperata, Morinda lucida, Jatropha gossypiifolia, Ocimum gratissimum*, and *Acalypha wilkesiana* against various bacterial pathogens of poultry, such as *Salmonella* spp. and fungal species, using a crystal violet plate microdilution method. The organic extract of *M. lucida* showed good antibiofilm activity (> 50% inhibition) against *Salmonella* Cholerasuis, *Salmonella* Idikan, *Salmonella* Kottbus and *S.* Enteritidis. Similarly, aqueous extracts of *M. lucida* also exhibited good antibiofilm activity (> 50% inhibition) against *Salmonella* Dublin, *S.* Idikan, *S.* Kottbus, and *S.* Typhimurium. The findings of this study provide researchers and chicken breeders with useful information on the use of additives of herbal origin (Olawuwo, Famuyide, McGaw, 2022).

The study by Johnson *et al*. (2022) examined the antimicrobial and antibiofilm activity of a chitosan-edible coating combined with *M. piperita* L., *Citrus limon*, *Ocimum sanctum Linn*. and *Plectranthus amboinicus* [Lour.] Spreng to control foodborne pathogens like *Salmonella* spp. present in chicken. The extracts of *M. piperita* and *P. amboinicus* exhibited a higher content of polyphenols, and when these were combined with chitosan, an inhibition of 90% of biofilm formation of *Salmonella* spp. was seen at ½ concentration of leaf extract with chitosan, while ¼ concentration reduced biofilm formation to 85%. These combinations controlled bacterial growth during a storage period of 15 days, demonstrating that edible coating with phenolic-rich extracts can prolong the shelf life of chicken during refrigerated storage and serve as a substitute for chitosan preservatives (Johnson *et al*., 2022).

Medicinal plants also have effects in inhibiting *Salmonella* biofilm formation, as demonstrated by Mulat, Khan and Pandita (2021) with Indian medicinal plants and by Erhabor *et al*. (2022) by many South African medicinal plants.

Plant derived-compounds

Among plant-derived compounds, the phenolics, especially flavonoids, are extensively researched for their antibacterial, anti-QS, and antibiofilm properties.

The effect of quercetin, a bioactive compound found in a variety of vegetables, on *Salmonella* biofilms, was investigated by Kim *et al*. (2022) and Roy, Song and Park. (2022). Kim *et al*. (2022) observed the inhibitory activities of quercetin against *S.* Typhimurium and *S.* Enteritidis on plastic and rubber gloves, and chicken skin during biofilm formation. When quercetin (0–125 μg/mL) was supplemented, the inhibitory effect was 1.50–2.61 log CFU/cm^2. The inhibitory impact was observed by microscopies (field-emission scanning electron microscopy and confocal laser scanning microscopy). Quercetin also downregulated the expression levels of virulence (*avrA*, and *hilA*), stress response (*rpoS*), and quorum-sensing (*luxS*) genes. In the study by Roy, Song and Park (2022), quercetin inhibited the *S.* Typhimurium biofilm by disturbing cell-to-cell connections and inducing cell lysis, resulting in the loss of normal cell morphology, and changes in swarming and swimming motilities at sub-MIC. The authors concluded that quercetin could be used as an antibiofilm agent in the food industry.

Santos *et al*. (2021) investigated the effect of phenolic compounds on antimicrobial, anti-QS and anti-biofilm formation against foodborne pathogens. Curcumin (1.5-6 µM) and capsaicin (250-1000 µM) at sub-MIC partially inhibited the biofilm formation by *S*. Montevideo. According to the authors, at high concentrations (above the MIC), phenolic compounds can exhibit antimicrobial activity, encouraging applications in the food and pharmaceutical industries.

As the text suggests, natural products including plant extracts and plant isolated compounds offer a promising and sustainable way to control microbial growth in the food industry. Plant extracts are rich in several kinds of bioactive compounds such phenolic compounds, essential oils and terpenoids which present antimicrobial properties able to inhibit or slow down the growth of spoilage and pathogenic organisms. In practical terms, these compounds can be applied to packing materials, used as natural preservatives or even used directly as edible coatings to the surface of foods (Pinto, Ayala-Zavala, 2024; Oulahal, Degraeve, 2022). These applications may help reduce the use of synthetic preservatives aligning with consumer expectations for clean labels and environmentally friendly products (Pinto *et al*., 2023).

A few examples can be highlighted on the practical use of natural products in the food sector including the use of phenolic enriched plant extracts as edible coatings to avoid microbial development and increase the shelf life of fresh cut vegetables as well as in meat and meat products (Pinto *et al*., 2023; Lima *et al*., 2022; Papuc *et al*. 2017). Active packaging materials enriched with bioactive compounds may also offer a protective barrier against microorganisms (Alonso, Fernández-Pastor, Guerrero, 2024; Oulahal, Degraeve, 2022). In fact, the use in edible coating and packaging materials seem to be the most promising applications of natural compounds, particularly considering that these compounds tend to lose activity when applied to complex systems such as food matrices. One of the ways to improve their stability and maintain their efficacy is by using micro or nano encapsulated formulations which could expand their applications to be included in cleaning and disinfecting solutions for use at industrial surfaces (Pinto, Ayala-Zavala, 2024):

The mode of action of bioactive compounds is still not fully comprehended. It has been suggested that phenolic compounds act by modifying the permeability of the membrane, inactivating intracellular enzymes, modifying the intracellular pH, interfering with generation of energy (ATP) and inhibiting DNA synthesis (Pinto *et al*. 2023; Bourab *et al*. 2019).

Another issue that needs to be further investigated is the potential for sensory interference by bioactive compounds in foods as some plant extracts and/or specific molecules may negatively affect the taste, texture, aroma or the color of the food products in which they may be applied (Pinto, Ayala-Zavala, 2024). A thorough safety evaluation, particularly with a focus on the toxicity of the extracts still need to be further explored. Even though several natural bioactive compounds or plant extracts that contain those have proven antimicrobial properties, their cytotoxic potential have not been evaluated to the same extent. Additionally, the dosage, time of exposure and composition of these extracts may affect their safety. Detailed toxicological studies are needed in order to define safe concentrations and avoid unnecessary risks to consumers' health (Vilas-Boas, Pintado, Oliveira, 2021; Vettorazzi *et al*., 2020).

Finally, the development of sustainable production techniques for making these plant extracts is crucial, incorporating production technology that is both environmentally friendly and economically viable for application in large-scale production (Chemat, Vian, Cravotto, 2012).

TABLE I - Overview of studies reporting inhibition of *Salmonella* biofilms with essential oils (EOs), plant extracts and plant-derived compounds

Form	**Plant/ compound**	***Salmonella* serotypes**	**Effects on biofilm (main findings)**	**Analytical methods**	**References**
Essential oil (EO)	Carvacrol, thymol and eugenol.	12 strains of *S.* Typhimurium	Compounds showed synergism with nalidixic acid with a reduction in biofilm eradication	Scanning electron microscopy (SEM)	Miladi *et al*. (2017)

EO	Carvacrol and thymol	*S.* Typhimurium, *S.* Enteritidis, *S.* Saint Paul	Both compounds inhibited biofilm, mainly carvacrol in *S.* Typhimurium (5 log CFU/cm²); and thymol in *S.* Enteritidis (4 log CFU/cm²)	Scanning electron microscopy (SEM)	Amaral *et al*. (2015)
EO	EO of peppermint (*Mentha piperita)* and lemongrass (*Cymbopogon citratus*)	*Salmonella* Enteritidis S64	Disinfectant solutions formulated with EO significant reduced (10 min) or eliminated (40min) biofilm	Plate count during biofilm formation, after swabs in coupons	Valeriano *et al*. (2012)
EO	*Allium sativum* and *Cuminum cimynum*	*Salmonella* Typhimurium	Reduced the amount of *Salmonella* Typhimurium in mature biofilm; Reduced expression of QS (*sdiA* and *luxS*) and cellulose synthesis genes (*csgD* and *adrA*)	Microplate crystal violet staining assay; Scanning electron microscopy (SEM) experiments; Realtime-quantitative PCR (RT-qPCR)	Hakimi Alni, Ghorban, Dadmanesh (2020)
EO	Clove; key compound: eugenol	*Salmonella* Enteritidis	Initial cell adhesion at MIC was inhibited by 49.8%	Microplate treated with crystal violet	Somrani *et al*. (2022)
EO	Cinnamon, thyme, clove, curcuma,	Multidrug-resistant *Salmonella*	Inhibition of biofilm formation by EO of cinnamon (99%), clove	Microplate treated with crystal violet	Alibi *et al*. (2022)

	rosemary, and sage	Enteritidis clinical strains	(98%), tyme (96%), rosemary (80%), curcuma (29%), sage (15%)		
EO	15 EOs from *Lippia origanoides*	*Salmonella* Enteritidis ATCC 13076 and *Salmonella* Typhimurium ATCC 14028	Biofilm inhibition (> 65%) on both microorganisms. EO inhibited the expression of QS-related genes (*luxR*, *luxS*, *qseB*, *sdiA*) and biofilm formation (*csgA*, *csgB*, *csgD*, *flhD*, *fliZ*, *motB*)	Microplate treated with crystal violet; SEM images; RT-PCR	Guillín *et al*. (2021)
EO	EO from *Salvia officinalis* L leaves	*S. enterica* from raw milk	EOs inhibited the biofilm formation of *S. enterica* isolates	Microplate treated with crystal violet	Selim *et al*. (2022)
EO	EO from *Elletaria cardamomum:* *α*-terpinyl acetate, 1,8-cineole, linalool acetate, sabinene.	*Salmonella* Typhimurium JSG 1748	Biofilm inhibition up to 100% at 0.125% of green cardamom EO	Microplate treated with crystal violet	Abdullah *et al*. (2021)
EO	EO from *Artemisia dracunculus;* key compound: estragole -	*Salmonella* Typhimurium	Inhibition and disruption of biofilm at sub-MIC	Microplate treated with crystal violet; SEM images; RT-PCR	Pelarti *et al*. (2021)

	methyl chavicol				
EO	Thyme (*Thymus vulgaris* L.) EO	*Salmonella* Typhimurium	EO inhibit biofilm formation in a dose-dependent concentration. At 10 µL/mL, the inhibition was 30%. At 100 µL/mL, the inhibition achieves 90%.	Microplate treated with crystal violet	Sateriale *et al.* (2023)
EO	*Rosa damascena* EO	*Salmonella enterica*	Minimal biofilm inhibition concentration of EO: MIBC50 equal to 0.270 mg/mL; MIBC90 equal to 0.291 mg/mL.	Microplate treated with crystal violet. MALDI-TOF was used to detect the biofilm development	Verešová *et al.* (2024)
EO	EO of cinnamon, garlic and onion	*Salmonella* Enteritidis	In the initial cell adhesion, the higher inhibition was by cinnamon EO (75% at 2 MIC). The eradicative effect was higher at 2 MIC for all EOs.	Microplate treated with crystal violet: initial attachment and eradication of preformed biofilms	Somrani *et al.* (2024)
Plant extracts	Burdock (*Arctium lappa* L.) leaves	*S.* Typhimurium	Inhibition of biofilm formation reached 70% with a concentration of 2.0 mg/mL of extracts	Microplate treated with crystal violet	Lou *et al.* (2016)
Plant extracts	*Ginkgo biloba* extract	*S.* Enteritidis	Antibiofilm activity was concentration dependent and inhibited biofilm formation	Microplate treated with crystal violet and glass slide under fluorescence	Wu *et al.* (2016)

				microscope	
Plant (leaves) extracts	*Alchornea laxiflora*, *Ficus exasperata*, *Morinda lucida*, *Jatropha gossypiifolia*, *Ocimum gratissimum*, *Acalypha wilkesiana*	*S.* Cholerasuis, *S.* Idikan, *S.* Kottbus, *S.* Enteritidis, *S.* Dublin, *S.* Idikan, *S.* Kottbus, *S.* Typhimurium	Biofilm inhibition (> 50%) by organic and aqueous leave extracts	Microplate treated with crystal violet	Olawuwo, Famuyide, McGaw (2022)
Plant extracts	*Mentha piperita* and *Plectranthus amboinicus* combined with chitosan	*Salmonella* spp.	Strong inhibition of biofilm of *Salmonella* spp. by sub-MIC	Microplate treated with crystal violet	Johnson *et al*. (2022)
Plant extracts	*Gloriosa superba* and *Solanum nigrum*.	*Salmonella* Typhimurium	Extracts inhibited biofilm formation	Microplate spectroscopic reading	Mulat, Khan, Pandita (2021)
Plant extracts (South African plants)	*Carpobrotus edulis*, *Vachellia rehmanniana*, *Vachellia xanthophloea*, *Kigelia*	*Salmonella* Typhimurium, *Salmonella* Enteritidis	All extracts inhibited biofilm formation completely or partially of *Salmonella* Typhimurium; Methanol extract of *V. xanthophloea* inhibited	Microplate treated with crystal violet	Erhabor *et al*. (2022)

	africana, Elephantorrhiza elephantina, Ochna pretoriensis		biofilm of *Salmonella* Enteritidis (77%)		
Plant-derived compound – Flavonoid	Quercetin	*Salmonella* Typhimurium and *Salmonella* Enteritidis	Biofilms supplemented with quercetin (125 µg/mL) grew thinner, looser, and were easier to eliminate. Quercetin downregulated the expression levels of virulence (*avrA, hilA*), stress response (*rpoS*), and QS (*luxS*) genes	Microplate treated with xylose-lysine-deoxycholate (XLD); Field-emission scanning electron microscopy (FE-SEM); RT-PCR	Kim *et al.* (2022)
Plant-derived compound: Flavonoid	Quercetin	*Salmonella* Typhimurium	Inhibition of biofilm and change the cell morphology; inhibited swarming and swimming motilities. Quercetin downregulated gene expression (*avrA, hilA, rpoS, luxS)*	Microplate treated with XLD; FE-SEM; RT-PCR	Roy, Song, Park (2022)
Plant-derived compound	Curcumin and capsaicin	*Salmonella* Montevideo	Curcumin (1.5-6 µM) and capsaicin (250-1000 µM) partially inhibited the biofilm formation by *S.* Montevideo	Microplate treated with crystal violet	Santos *et al*. (2021)

CONCLUSIONS

This review summarized the important aspects of biofilm formation, with a focus on *Salmonella*. Additionally, we showed the importance of EOs, plant extracts, and specific compounds in effectively inhibiting biofilm formation by *Salmonella* spp. Studies involving these sources are becoming increasingly necessary due to the inherent tolerance of bacterial biofilms and the rise in antimicrobial resistant organisms.

Of note, most studies evaluating the use of plant-derived compounds have been carried out *in vitro*, using only one strain in a monoculture, in addition to using one type of surface, in specific conditions of culture medium, temperature, and atmosphere. In addition, the analytical methods that have been used normally are not able to specifically pinpoint the mechanism of action of these compounds on biofilm inhibition. Another issue that deserves attention is the fact that EOs and plant extracts contain multiple compounds, making it hard to identify a specific molecule responsible for the observed effect. Novel studies should address these issues and involve multispecies or multi-strain biofilms, in addition to combining different antimicrobials in food matrices and industrial relevant conditions.

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AUTHORS’ CONTRIBUTIONS

Beatriz X. V. Quecán: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Visualization. Emília M. F. Lima: Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. Felipe A. Almeida: Investigation, Writing – original draft, Writing – review & editing, Visualization. Vanessa Bueris: Investigation, Writing – original draft. Ali Tahrioui: Writing – review & editing.

Uelinton M. Pinto: Conceptualization, Writing – review & editing, Supervision, Project administration, Resources.

CONFLICT OF INTEREST STATEMENT

The authors declare that this review was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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