

Dissecting the Phenotypic Complexity of Biofilm Development and Quorum Sensing in *Salmonella enterica* Serovars

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2 Sensing in *Salmonella enterica* Serovars

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25 Abstract

26 This study investigates the mechanisms of biofilm formation and quorum sensing in *Salmonella enterica* serovars, with particular
27 emphasis on their implications for clinical safety and public health. The ability of this bacterium to form biofilms on various

surfaces poses significant challenges for control and eradication efforts, contributing to persistent infections and enhanced antimicrobial resistance. The study explores quorum quenching strategies to inhibit biofilm formation through certain treatments. The research employs crystal violet staining, bacterial growth curve analysis, and fluorescence microscopy to evaluate the effects of quorum sensing (QS) and natural product-based quorum quenching strategies on biofilm formation on abiotic (polystyrene) surfaces. Short- to medium-chain acyl-homoserine lactones (AHLs), particularly C8-HSL were assessed for their role in promoting biofilm development, alongside plant-derived and dietary natural products with reported antimicrobial activity, including aloe emodin (from *Aloe vera*), epigallocatechin gallate (EGCG) (from green tea, *Camellia sinensis*), thymoquinone from (*Nigella sativa*) garlic extract (active compound *Allium sativum*), and turmeric extract (active extract *Curcuma longa*). Results demonstrated that C8-HSL significantly enhanced biofilm formation (Figures 2–4; Tables 1–2), with 0.2 mM identified as the most effective concentration (Figure 4). In contrast, the tested natural products exhibited quorum quenching and anti-biofilm activity, significantly reducing biofilm biomass and structural integrity (Figures 5 and 6). Fluorescence microscopy further supported these findings by demonstrating enhanced biofilm formation in the presence of quorum sensing molecules and disrupted biofilm architecture following quorum quenching treatment (Figures 7 and 8). These findings highlight the central role of quorum sensing in *Salmonella enterica* pathogenicity and demonstrate the potential of plant-derived and dietary natural products as alternative quorum quenching agents for biofilm control. The study suggests that further research, including the use of genetically engineered mutants, is required to elucidate the specific genetic pathways involved in QS-mediated biofilm regulation. Overall, this research advances understanding of biofilm formation and quorum sensing in *Salmonella enterica* and supports the development of novel strategies to manage persistent infections and improve food safety.

Key Words: N-acyl homoserine lactone (AHL), *Salmonella* Typhimurium 14028, *Salmonella* Typhimurium Wildtype and *Salmonella* Enteritidis UCH, *Salmonella* Virchow, C8HSL, Farnesol, CV assay, fluorescence microscopy, antibiotic susceptibility test, Biofilm formation, quorum sensing, quorum quenching and natural compounds.

1. Introduction

Salmonella is a major causative agent of foodborne illness, specifically known as salmonellosis, and is responsible for an estimated 93.8 million intestinal infections and 155,000 deaths annually, ranking third in the global mortality index. Estimated 80.3 million cases were food borne (Roasto et al., 2023). According to the European Food Safety Authority (EFSA), *Salmonella* remains a significant public health concern due to its ability to cause food poisoning via contaminated food (EFSA and ECDC, 2021). The EU One Health Zoonoses report identified salmonellosis as the second most frequently reported gastrointestinal infection in the European Union. Recent studies have revealed a strong link between *Salmonella* pathogenesis and biofilm formation, highlighting a significant correlation between the two (EFSA and ECDC, 2021) (Pradhan et al., 2023). This relationship between quorum sensing, biofilm development and virulence in *Salmonella* has been comprehensively reviewed, emphasising how inter-bacterial signalling enhances pathogenicity (Sholpan et al., 2021). During biofilm development, bacteria secrete extracellular polymeric substances (EPS) to form a protective matrix. These EPS-enclosed biofilms with different types of bacteria can adhere to both abiotic (non-living) and biotic (living) surfaces (Jabłońska-Trypuć et al. 2022).

Biofilms confer resistance to antibiotics, antimicrobial agents, and harsh environmental conditions, such as exposure to heavy metals, as well as protection from the host immune response. This makes it especially challenging to eliminate foodborne pathogens like *Salmonella* shown to persist and contaminate eggs, meats and packaged foods (Roasto et al., 2023) (Liu et al., 2024). These biofilm matrices are regulated by a complex and intricate form of cell-to-cell communication known as quorum sensing, which occurs through

signalling molecules called autoinducers (Aslam et al., 2023). *Salmonella* biofilm formation can be influenced by different classes of signalling molecules present in the gastrointestinal environment: AI-1, AI-2, AI-3, indole and diffusible signal factors (DSF) (Sholpan et al., 2021; Aslam et al., 2023; Coquant et al., 2021). Quorum-sensing signalling molecules regulate the expression of virulence factors that contribute to disruption of the host intestinal barrier, facilitating the establishment of persistent infections (Cheng et al., 2024). Interestingly, *Salmonella* does not produce AHLs itself but can detect these signals from other microorganisms through the SdiA protein as a quorum sensing receptor, a LuxR homolog (Sholpan et al., 2021; Aslam et al., 2023).

Upon detection, *Salmonella* cells interpret these signals and execute specific cellular responses. AI-2 is a diffusible signalling molecule produced via the LuxS enzyme in *Salmonella* and *Escherichia coli*, facilitating interspecies quorum sensing communication (Xie et al., 2024; Hetta et al., 2024). Homologues of the *luxS* gene are widely distributed across bacterial species, and extracellular AI-2 activity has been detected in diverse LuxS-producing organisms, supporting its role in interspecies signalling (Hetta et al., 2024). In *S. Typhimurium*, AI-2 production is growth-phase dependent, with increased accumulation during active growth and reduced signalling in stationary phase (Xie et al., 2024). Disruption of the *luxS* gene impairs quorum sensing and has been associated with altered biofilm formation and virulence-related gene expression, highlighting the importance of AI-2 signalling in biofilm regulation (Xie et al., 2024; Liu et al., 2025). AHL-producing bacteria have been shown to enhance *Salmonella* biofilm formation both within the host and on abiotic surfaces (Blana et al. 2017) (Almeida et al. 2017). The risk posed by *Salmonella* serovars to public health is closely linked to their capacity for biofilm formation (Pradhan et al., 2023). Recent evidence shows that long-chain AHLs such as C12-HSL can significantly stimulate biofilm formation in *S. Enteritidis* under anaerobic conditions, accompanied by increased *adrA* expression, a key regulator of cyclic-di-GMP signalling (Almeida et al., 2017). This trait equips them to persist on a wide range of surfaces encountered by humans, including food-contact surfaces such as metals, plastics, hospital equipment, and organic materials (Jabłońska-Trypuć et al., 2022; Roasto et al., 2023). Therefore, it is essential to elucidate the unknown mechanisms of biofilm formation and identify the genetic factors responsible.

Experimental studies have demonstrated that exogenous quorum sensing molecules can significantly enhance *Salmonella* biofilm formation. For example, Campos-Galvão et al. (2015) reported that short-chain quorum sensing molecules (C6–C12) increased biofilm formation by *Salmonella Enteritidis* PT4 under both nutrient-rich and minimal conditions. Specifically, C12-HSL influenced the expression of genes associated with biofilm formation and cell attachment. Similarly, Solano et al. (2001) highlighted the role of extracellular matrix components in *Salmonella* biofilm development, contributing to surface attachment and persistence. Supporting these findings, Almeida et al. (2017) demonstrated that exogenous AHLs (C6-HSL, C8-HSL and C12-HSL) enhance anaerobic biofilm formation in *S. Enteritidis*. This is consistent with broader evidence showing that *Salmonella* can detect AHLs produced by other bacterial species and modulate biofilm-associated gene expression through quorum sensing mechanisms (Zhang et al., 2022).

However, considering the complexity of the gastrointestinal tract's bacterial flora, the role of quorum sensing, especially the involvement of AHLs produced by other bacteria, requires further investigation (Wu et al., 2024). The development of quorum-sensing inhibitors (QSIs) and quorum-quenching (QQ) strategies represents a promising approach for reducing *Salmonella* infections and offers new perspectives for controlling bacterial virulence (Alum et al., 2025). Farnesol, often abbreviated as FAR, is a naturally occurring acyclic sesquiterpene alcohol from the isoprenoid family. Its role has been extensively studied in *Candida* species, where it acts as a QS molecule regulating fungal hyphal maturation (Shchepin et al.,

2003). The anti-biofilm activity of trans-trans farnesol (t-t farnesol) has been demonstrated in bacterial species such as *Staphylococcus epidermidis*, where it disrupts biofilm structure and matrix composition (Gomes et al., 2011). However, its biofilm-quenching effect against *Salmonella* Enteritidis and *Salmonella* Typhimurium remains unexplored. To date, limited attention has been given to the use and effectiveness of quorum quenchers in disrupting *Salmonella* biofilms. Understanding how *Salmonella* uses QS to regulate biofilms is a vibrant research area. *Salmonella enterica* produces the universal quorum-sensing molecule autoinducer-2 (AI-2) via the LuxS enzyme and participates in interkingdom signalling by detecting bacterial autoinducer-3 (AI-3) as well as host-derived catecholamine hormones, including epinephrine and norepinephrine, through two-component systems such as QseC/QseB and QseE/QseF (Hamed et al., 2022; Ding et al., 2025; Wu et al., 2024). Notably, *Salmonella* lacks its own acyl-homoserine lactone (AHL) synthase but possesses an orphan LuxR-type receptor, SdiA, which enables it to detect and respond to AHL signals produced by other microbial species (Sholpan et al., 2021; Zhang et al., 2022). Through SdiA, *Salmonella* can respond to AHLs (e.g., 3-oxo-C6-HSL, C8-HSL and C12-HSL) produced by co-occurring microbial species, leading to changes in gene expression associated with attachment and biofilm formation (Zhang et al., 2022; Sholpan et al., 2021; Almeida et al., 2017).

These signalling pathways converge on regulatory networks that control biofilm-associated genes. AI-2 signalling regulates the *lsr* operon and influences the expression of genes involved in motility, fimbrial adhesion, and *Salmonella* pathogenicity island (SPI) functions (Zhang et al., 2022; Sholpan et al., 2021; Li et al., 2025). Similarly, SdiA in *S. Typhimurium* activates the *rck* operon located on the virulence plasmid in response to AHL signals, leading to the expression of factors involved in adhesion and host cell invasion, as demonstrated in earlier but foundationally relevant studies (Smith and Ahmer 2003; Abed et al., 2014). Quorum sensing also interacts with core cellular processes; recent studies indicate that LuxS/AI-2 signalling can modulate stress responses and motility, linking environmental adaptation to collective bacterial behaviour (Zhang et al., 2022; Li et al., 2025). In *Salmonella enterica*, quorum sensing is increasingly recognised as a regulator of biofilm lifecycle dynamics, influencing processes such as motility, adhesion, and matrix-associated gene expression. These coordinated responses are thought to contribute to transitions within the biofilm, including dispersal, although the underlying mechanisms remain less well defined compared to other Gram-negative bacteria (Avcı et al., 2026; Afrasiabi and Partoazar, 2024; Bhuiyan et al., 2025).

Despite these advances, significant research gaps remain. The role of small non-coding RNAs in quorum sensing regulation in *Salmonella* is still poorly understood, despite sRNA-mediated quorum sensing control being well characterised in other bacteria such as *Vibrio* spp. Furthermore, the integration of metabolic state with quorum sensing, particularly given the dual role of LuxS as both a metabolic enzyme and signalling mediator, remains an important area for further investigation (Afrasiabi and Partoazar, 2024; Li et al., 2025; Zhang et al., 2022). Emerging trends in quorum sensing research include the study of non-canonical signalling molecules such as diffusible fatty acids (DSF) and indole, which contribute to interspecies communication and biofilm regulation (Wu et al., 2024; Afrasiabi and Partoazar, 2024; Bhuiyan et al., 2025). Interestingly, *Salmonella* exhibits altered behaviour in plant-associated environments. For example, interactions with *Pectobacterium carotovorum* in tomato soft rot systems have been shown to influence *Salmonella* physiology and signalling responses, highlighting the complexity of interspecies communication in these niches (Avcı et al., 2026) (George et al., 2018). However, its growth was significantly enhanced in soft-rotted tomato tissue, suggesting that such conditions are favourable for *Salmonella* proliferation (Avcı et al., 2026) (George et al., 2018).

It remains unclear whether plant compounds are directly interacting with or binding to the SdiA protein, a possibility proposed by. This potential interaction will be discussed further in the section on antimicrobial and quorum sensing inhibition strategies. Additionally, microbial signals such as indole have been shown to negatively regulate *Salmonella* virulence, including suppression of virulence-associated pathways and modulation of host–pathogen interactions (Ferrer et al., 2024; Fan et al., 2024; Franco et al., 2024). Exploring these non-conventional quorum sensing interactions may offer new insights into how *Salmonella* fine-tunes its biofilm formation and pathogenicity. A major limitation of conventional antimicrobial strategies is that biofilm-associated infections and surface contamination are often tolerant to antibiotics and disinfectants, and selective pressure can accelerate resistance. An alternative approach is to attenuate virulence and biofilm development by interfering with quorum sensing (QS) and QS-linked phenotypes (motility, adhesion, EPS production, curli/fimbriae expression), rather than relying solely on bactericidal action. Natural products are attractive candidates for this strategy because they often contain multi-target bioactives that can disrupt attachment, matrix integrity, motility, and signal-regulated gene expression, while also being relevant to food/clinical environments.

Natural product-derived quorum sensing inhibitors (QSIs) have gained attention as anti-virulence approaches to control biofilm formation without necessarily imposing strong bactericidal selective pressure. Plant-derived compounds, including polyphenols, essential-oil constituents, organosulfur compounds, and anthraquinone derivatives, are increasingly recognised as quorum-sensing inhibitors (QSIs) and anti-biofilm agents. These compounds have been reported to disrupt adhesion, motility, matrix integrity, and quorum sensing-regulated gene expression in bacterial systems (Alum et al., 2025; Afrasiabi and Partoazar, 2024; Fan et al., 2024; Bhuiyan et al., 2025). In this study, we selected a structure-diverse panel of natural products relevant to dietary and botanical sources: epigallocatechin gallate (EGCG) from green tea (*Camellia sinensis*), thymoquinone from (*Nigella sativa*), garlic extract from *Allium sativum*, turmeric extract/curcumin from *Curcuma longa*, and the anthraquinone aloe emodin associated with *Aloe* spp. Epigallocatechin gallate (EGCG) has been reported to attenuate *Salmonella* virulence phenotypes and reduce biofilm formation, with evidence suggesting involvement of quorum sensing-related pathways, supporting its use as a strong comparator compound (Cheng et al., 2024; Fan et al., 2024). thymoquinone and other essential-oil constituents have demonstrated anti-biofilm activity against *Salmonella*, including on food-contact surfaces, and may influence biofilm-associated processes such as adhesion and motility (Wang et al., 2023; Soni et al., 2013). Curcumin has been reported to exhibit anti-biofilm activity and interfere with quorum sensing systems, leading to reduced adhesion and virulence-associated traits in bacterial pathogens, including *Salmonella* (Packiavathy et al., 2014; Tyagi et al., 2015). Garlic-derived organosulfur compounds, including allicin and diallyl disulfide, have been shown to interfere with quorum sensing systems in Gram-negative bacteria by modulating QS-regulated gene expression and signalling pathways, supporting their use as potential broad-spectrum quorum-quenching agents (Alum et al., 2025; Zhang et al., 2024). Finally, aloe emodin represents an anthraquinone scaffold with documented antimicrobial and anti-biofilm activity across a range of bacterial species, supporting its evaluation for potential modulation of *Salmonella* biofilm formation within a natural-product screening framework (Qun et al., 2023; Li et al., 2021).

Note: Underlined labels represent genes yet to be fully explored. Circled genes are directly relevant to this research.

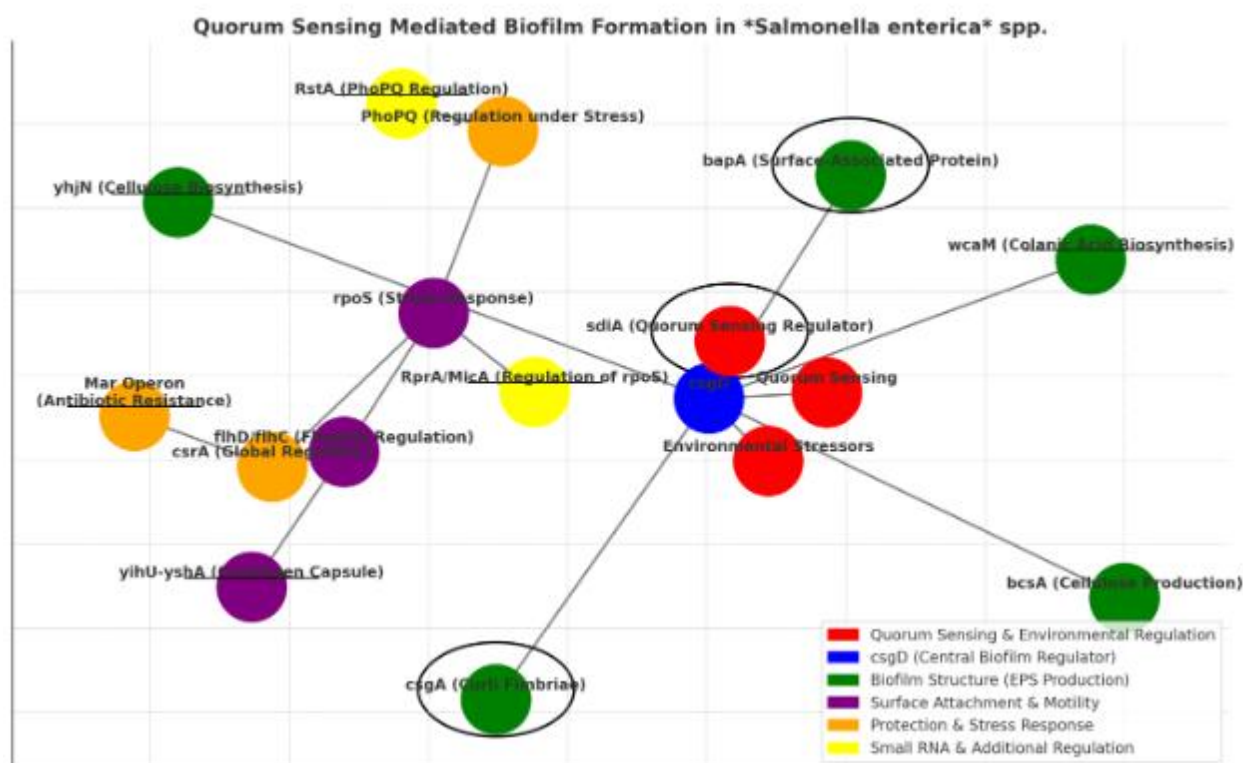


Fig 1. Genetic map overview (using salmonet2) (Olbei et al 2022)

This genetic map illustrates the key regulatory interactions governing biofilm formation in *Salmonella enterica* serovars. The map highlights how quorum sensing and environmental stressors regulate the central biofilm regulator and how they work together, which in turn activate genes involved in biofilm structure, surface attachment, motility, protection, and stress response. This map provides a visual representation of the genetic network controlling *Salmonella* biofilm formation. It serves as a tool for understanding how *Salmonella* persists in hostile environments, contributing to chronic infections, food contamination, and antibiotic resistance. The map was created using salmonet2 (Olbei et al 2022)

Therefore, a deeper understanding of the role of signalling molecules, especially AHLs, in the development and maturation of *Salmonella* biofilms will enhance our ability to manage biofilm-associated persistent infections, more specifically, biofilm formation in presence of quorum sensing molecules on the enteric cell lines, Caco -2, will give an insight on bacterial behaviour.

Investigating quorum quenchers such as farnesol in the context of *Salmonella* biofilm formation on Caco-2 cells will further inform the development of novel preventive strategies targeting biofilm formation on living surfaces. The objective of this study is to explore this under-researched area by examining the concentration-dependent effects of quorum-sensing molecules on *Salmonella* biofilm development on Caco-2 cell lines, as well as the impact of quorum quenchers on biofilm disruption. The findings of this study are expected to contribute substantially to the existing body of knowledge regarding the role of quorum-sensing molecules in biofilm formation. The findings demonstrate that bacterial biofilm formation is contingent upon the presence of specific, preferential quorum-sensing molecules, and that bacterial behaviour differs significantly between in vivo and in vitro environments. Moreover, the data suggest that farnesol exhibits biofilm-disruptive properties, underscoring its potential as an anti-biofilm agent. These

insights hold significant implications for the development of therapeutic strategies aimed at mitigating persistent *Salmonella* infections. Furthermore, the outcomes may inform improvements in food safety protocols by aiding in the reduction of food spoilage and contamination risks. The broader significance of this research lies in its potential to enhance understanding of bacterial pathogenicity, host-pathogen interactions, and the molecular mechanisms underlying biofilm development, thereby facilitating the advancement of novel approaches to address biofilm-associated challenges.

2. Materials and Methods

2.1 Bacterial Strains and Culture Conditions

Salmonella enterica serovar Virchow (NCTC 7437), *Salmonella* Typhimurium TA1537, were obtained from the University of Westminster culture repository. Strain *Salmonella enterica* serovar Typhimurium Wild Type (ATCC 14028) was obtained from ATCC. Strain identities were confirmed by API20E biochemical profiling and whole-genome sequencing. The former two *S. Typhimurium* are plasmid-less species, and the later contains a large plasmid of 90 kb, called pSLT. The bacterium *Pseudomonas aeruginosa* POA1 and *Escherichia coli* NCTC 10418 were obtained from the University of Westminster repository and used as positive and negative controls for biofilm formation studies. The bacterial strains were maintained on Luria-Bertani (LB) agar plates at 37°C. The isolates were identified using conventional methods and the API 20E System (BioMerieux, France), supplemented with whole genome sequencing (WGS) through the microbes NG sequencing service. All the bacterial serovars were subjected to antimicrobial resistance testing. Serotyping was conducted using anti-O and anti-H antisera. *Salmonella* colonies were cultured in LB broth at 37°C overnight at 150 RPM in an INNOVA42 shaking incubator (15282117) for 24 hours. The culture was subsequently diluted to an optical density (OD) of 1.0 (approximately 10^7 CFU/mL) at 570 nm for further use.

2.2 Quorum sensing, quorum quenching molecules and natural products.

N-hexanoyl-L-homoserine lactone (C6HSL), N-butyryl-L-homoserine lactone (C4HSL) and [N-\(3-Oxo-octanoyl\)-L-homoserine lactone](#) (C8HSL) from Cayman Chemical Company (1.800.364.9897), N-3-Oxo-dodecanoyl L-homoserine Lactone” (3-oxo-C12HSL), Sigma Aldrich (09024-10mg) were used as quorum sensors and Farnesol 95% (C15H26O) Sigma Aldrich (14kx2826v) as quorum quencher. A stock solution of 1 mM was prepared for each quorum signaling molecule in ethyl acetate, and further dilutions to 0.2 mM, 0.05 mM, and 0.01 mM were prepared by serial dilutions from the 1 mM solution. All other chemicals were of analytical grade.

Epigallocatechin gallate (EGCG), aloe emodin, and (used as a thymoquinone -related bioactive compound), together with other test compounds, were obtained from Sigma-Aldrich (Merck, UK). Supplier details, catalogue numbers, lot/batch numbers, and CAS numbers are provided in Supplementary Table X.

Test compounds batch numbers Specifically, epigallocatechin gallate (catalogue no. E4268; CAS no. 989-51-5), aloe

emodin (catalogue no. A7687; CAS no. 481-72-1), and thymoquinone (catalogue no. 274666; CAS no. 490-91-5) were used as representative natural bioactive compounds. All reagents were of analytical grade and were stored according to the manufacturer's recommendations prior to use. Each compound was prepared as a concentrated stock solution in analytical-grade ethyl acetate. Briefly, accurately weighed quantities of each compound were transferred into sterile amber glass vials and dissolved in ethyl acetate to obtain stock concentrations of 2 mg/ml. Samples were vortexed and sonicated where required to ensure complete dissolution. Insoluble material was removed by centrifugation, and the clarified supernatants were retained for further use. Working solutions were prepared by diluting stock solutions tenfold in ethyl acetate to achieve a final concentration of 200 µg/ml. Dilutions were performed under sterile conditions immediately prior to experimental use. Equivalent volumes of ethyl acetate without test compounds were used as solvent controls in all assays. Prepared solutions were stored at 4 °C in the dark and used within a defined period to minimise degradation and loss of bioactivity.

2.3 Growth curve

For initial growth curve experiment multiple bacterial species (*E. coli* NCTC 10418, *Pseudomonas aeruginosa* POA1, *Salmonella* Virchow, *Salmonella* Enteritidis, *Salmonella* Typhimurium Wild Type, *Salmonella* Typhimurium 14028 and *Salmonella* TA1537). All bacterial samples were grown in liquid medium following standard procedures. Different dilutions of the listed QSM were added to the tubes and incubated for 12 hours. Experiment results were plotted in Figure 2.

Single colonies of each *Salmonella* species were picked and inoculated into separate 15 mL tubes. Absorbance readings were taken every hour for 10 hours at 570 nm using a spectrophotometer. Four growth conditions were tested: one without a quorum sensing molecule (QSM) supplement, and a set of three supplemented with C4HSL, C6HSL, C8HSL, C12HSL and farnesol as a quorum quencher. Absorbance readings were recorded and analysed using GraphPad Prism to construct a bacterial growth curve over a 10-hour period, identifying the lag phase, log phase, stationary phase, and death phase. A semi-logarithmic graph was used, with the x-axis (time) set on a linear scale and the y-axis (optical density at 570 nm) set on a logarithmic scale. P values were assessed using a T-test between samples treated with QSM and without QSM at the 8-hour mark to calculate statistical significance. Values were plotted and analysed using GraphPad PRISM.

2.4.1 Biofilm Induction and Quorum Sensing Molecule Supplementation

Salmonella serovar isolates (*S. Enteritidis* and *S. Typhimurium* 14028) were cultured in 12-well plates containing LB broth and incubated at 37 °C for 24 hours to allow the bacteria to form biofilms. To evaluate the effect of quorum sensing molecules (QSMs), N-acyl homoserine lactones (HSLs), C4HSL, C6HSL, C8HSL, and C12HSL, were studied at three varied concentrations: 1 mM, 0.2 mM, and 0.05 mM.

Before QSM supplementation at the 24-hour time point, bacterial growth was assessed by measuring the optical density (OD) at 570 nm using a microplate reader. Following QSM addition, the plates were returned to incubation under the same conditions. All measurements were performed in triplicate across three independent plates under identical conditions to ensure experimental consistency. After 48 hours of incubation, OD at 570 nm was measured again to assess changes in bacterial growth in response to QSM exposure. Appropriate controls were included:

- Blank control: LB broth only
- Positive control: LB broth with bacterial inoculum
- Negative control: LB without QSMs

At the 72-hour mark, final OD readings at 570 nm were recorded to monitor continued bacterial growth.

2.4.2 Crystal Violet (CV) Assay for Biofilm Quantification

Following the final OD measurement at 72 hours, all 12-well plates were subjected to a crystal violet (CV) staining assay to quantify biofilm formation. Plates were gently shaken over a waste container to discard planktonic (free-floating) cells, preserving only the adherent cells attached to the well surface, representing biofilm biomass. Each well was then rinsed thoroughly with distilled water to remove remaining planktonic cells. At this stage, a portion of the biofilm was collected and stored for subsequent DNA and RNA extraction. To stain the biofilm, 500 μ L of 0.1% crystal violet solution was added to each well and incubated for 15 minutes at room temperature. After staining, wells were rinsed with distilled water to remove excess dye, ensuring uniform staining of biofilm without overstaining. The plates were then incubated at 60 °C for 1 hour to remove residual moisture. Once dry, 250 μ L of 33% acetic acid was added to each well to solubilise the bound dye. The plates were incubated for 10 minutes, after which the contents of each well were mixed thoroughly by pipetting up and down. The solubilised dye was transferred to a fresh 12-well plate to eliminate background interference from residual dye or biomass. Absorbance was measured at 570 nm using a microplate reader to quantify biofilm biomass.

2.4.3 Data Analysis

Data was exported to GraphPad Prism for analysis. Bar graphs were generated to compare OD₅₇₀ values across different quorum-sensing molecule concentrations for each *Salmonella* strain. Statistical significance was evaluated using one-way ANOVA, and *p*-values were calculated to determine the impact of each HSL concentration on bacterial growth and biofilm formation.

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2.5.3 CV 6-well microplate biofilm assay on Caco2 cell line: Fluorescence imaging

Fluorescence microscopy was used for visualization of the formation of *Salmonella* Enteritidis biofilms after the exposure of (C6HSL) and (C4HSL), on the Caco-2 surface. For this, bacteria and cells were cultured in a 6-well plate containing coverslips (22x22mm square) and treated with 0.05 and 0.2mM of C4HSL and C6HSL respectively. The coverslip from well 3 was removed and placed in a similar size petri dish, to observe separately. For cell staining two types of dye from (CellTracker™ Fluorescent Probes Pub. no. MAN0010620), USA) were used. Cell trackers green CellTracker™ Green CMFDA, 20 × 50 µg and red CellTracker™ Red CMPTX dyes, 20× 50 µg) (Invitrogen, USA). Each of these two staining dyes was added in 400µl concentration to each well of the 6-well plate, while 200µl of each dye was added into a Petri dish containing coverslip 3. The plate and petri dish were incubated at 37°C for 30 minutes. The culture medium was discarded from microtiter plates obtained as described above and washed twice with distilled water. The coverslips were transferred to slides using DAPI (4',6-diamidino-2-phenylindole)-Thermo Fisher Scientific. Solution and biofilms were observed under a fluorescence microscope to assess cell viability within the biofilm according to the manufacturer's protocol. The excess media from the 6-well polystyrene plate, which included the cover slip, was expeditiously eliminated, and the staining process was carried out prior to the cells becoming desiccated. A volume of 400 µl of the staining solution was meticulously transferred onto the biofilm sample on coverslip. The polystyrene plate was covered with aluminium foil and incubated for 30 minutes at ambient temperature, while being shielded from light exposure. The sample was gently rinsed with water that had been disinfected using a filter. The surplus stain was rinsed away using water applied from the lower portion of the support material. The cover slip was affixed utilising a 4% formaldehyde solution and thereafter examined under a fluorescence microscope with the aid of DAPI staining. The observations were made at magnifications of 40X and 20X.

2.6 Statistical analysis

The biofilm production by *Salmonella* Enteritidis in the presence of multiple AHLs was statistically analysed, utilising variance analysis (ANOVA), followed by a detailed evaluation via Tukey's multiple comparison test. In addition, the data derived from *Salmonella* Enteritidis biofilm growth over biological surfaces influenced by QS and QQ was subjected to two-way Variance Analysis (ANOVA), a p-value less than 0.005 was significant, employing Bonferroni's follow-up testing mechanism. Comparative data evaluations were undertaken utilising the GraphPad Prism Version 5.00 software for Windows (GraphPad Software, Boston, Massachusetts, USA).

3. Results

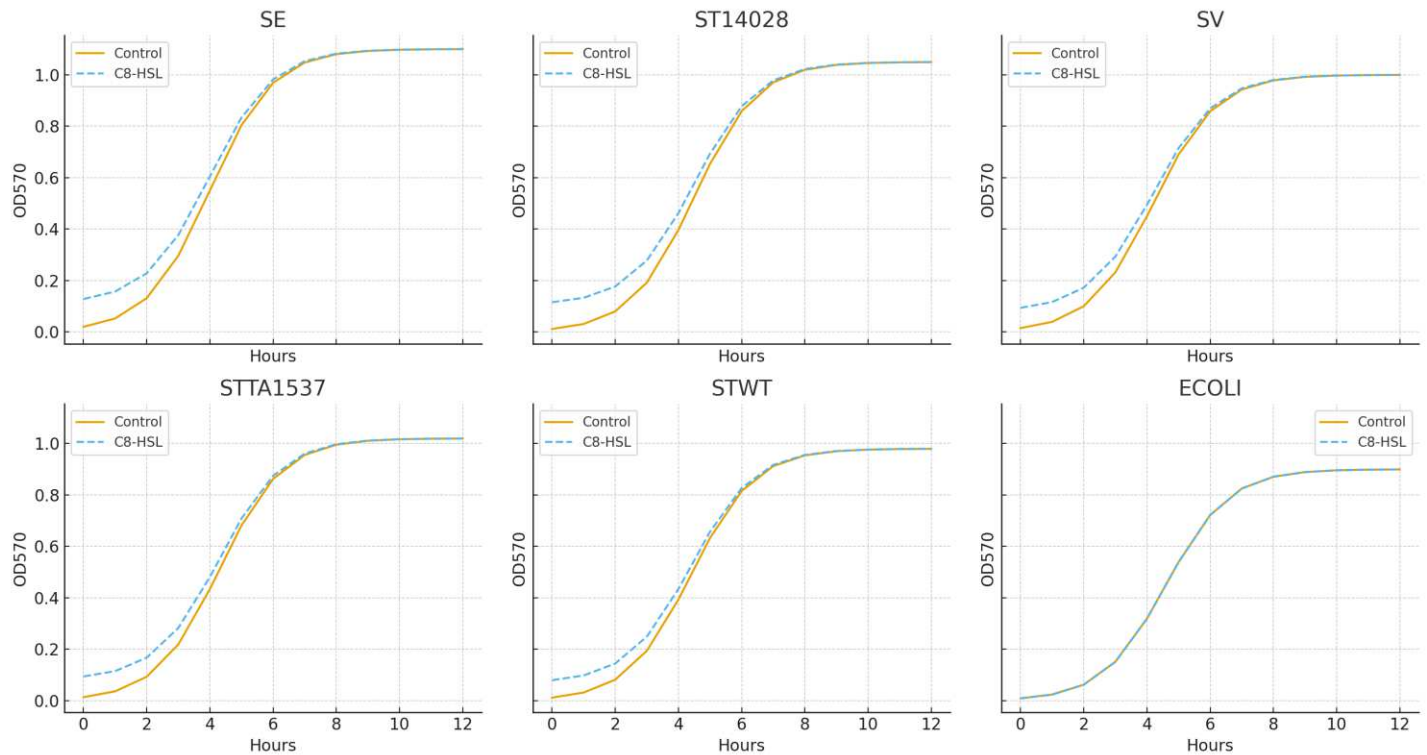
3.1 Quorum Sensing Molecules influences bacterial growth.

To determine whether exogenous quorum-sensing molecules affect bacterial proliferation, growth curves were generated for multiple bacterial species in the presence and absence of C8HSL (Figure 2). The strains tested included *Salmonella* Enteritidis (SE), *S. Typhimurium* 14028 (ST14028), *S. Virchow* (SV), *S. Typhimurium* TA1537,

353 *S. Typhimurium* wild type, *Escherichia coli*, and *Pseudomonas aeruginosa*. Across the *Salmonella* isolates, exposure
354 to C8HSL increased growth during the exponential phase relative to untreated controls (Figure 2). In contrast, *E. coli*
355 and *P. aeruginosa* showed little or no response to C8HSL treatment (Figure 2). Statistical comparison using two-
356 way repeated-measures ANOVA and area-under-the-curve (AUC) analysis confirmed significant C8HSL-associated
357 growth enhancement in all *Salmonella* isolates, whereas no significant effect was observed for *E. coli* or *P.*
358 *aeruginosa* (Table 1).

359 **Figure 2. Growth Curve multi-species comparison**

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361

362 **Figure 2 analysis** -The presence of C8HSL homoserine lactones shows overall increased exponential phases in all *Salmonella*
363 isolates compared to no treatment. The late exponential growth phase ended at around the 8–9-hour mark into the stationary
364 phase for the *Salmonella* isolates. This time frame was used for further experiments. *E. coli* and *Pseudomonas aeruginosa* did
365 not show any changes to exposure to C8HSL. *P* values displayed in the graphs were carried out using a one-way repeated
366 measure ANOVA plot to ensure statistical significance among groups, all of which displayed significance (*P* value <0.05) except
367 *Pseudomonas aeruginosa*. A two-way repeated measure ANOVA plot was used to compare treatment within species, and for
368 further investigation, AUC (area under the curve) averaged values were used to assess the significance of C8HSL's influence on
369 *Salmonella* isolates, all compared in Table 1 below.

370 **Table 1-** Statistical comparison of bacterial growth responses to C8HSL across bacterial species.

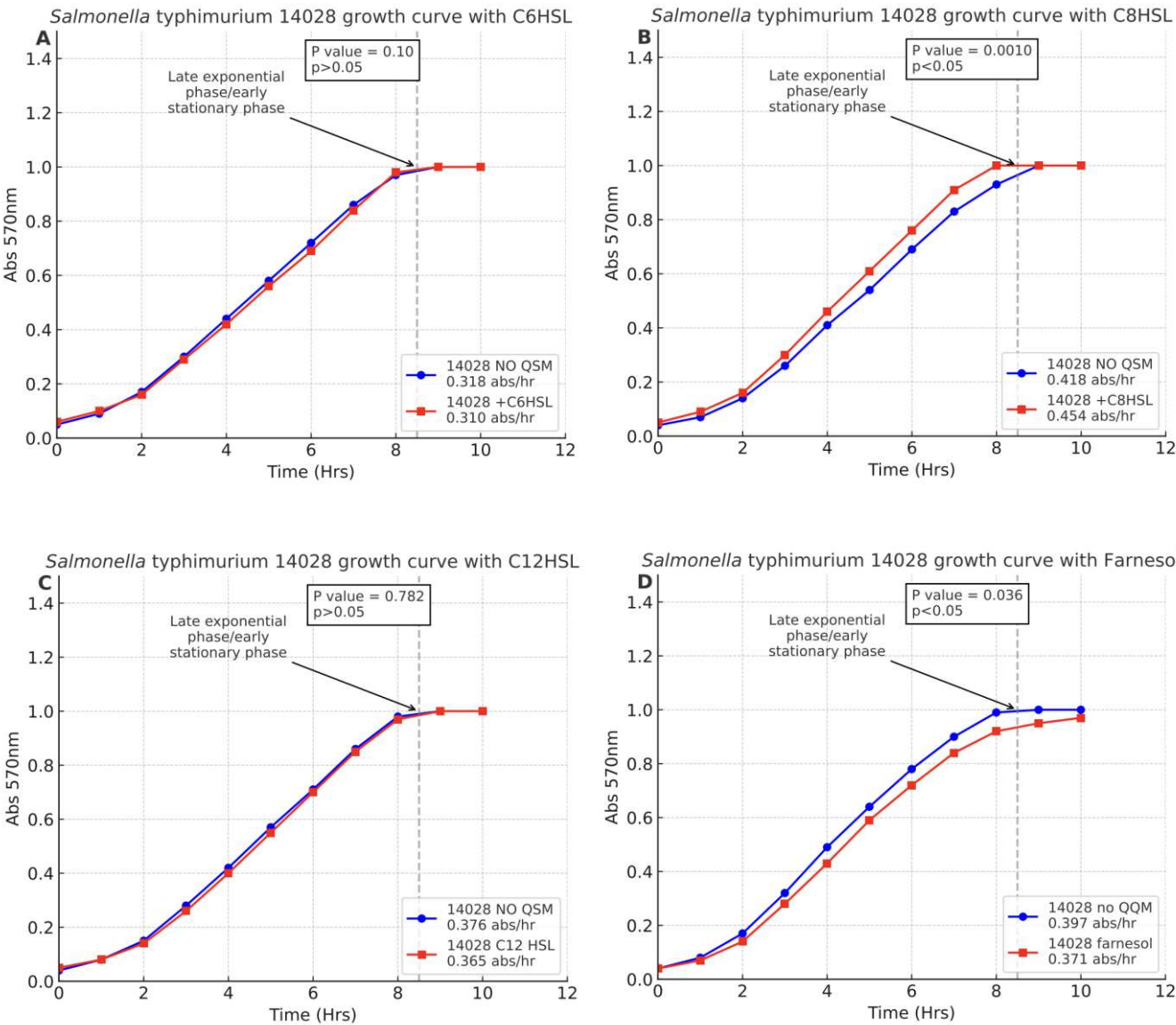
Species	P value using two-way RM-ANOVA	AUC (area under the curve) comparison	Analysis
<i>Salmonella</i> Virchow	SV vs SV(C8HSL)-0.0488	SV-6.488 /SV(C8HSL)-7.086	C8HSL positively

			increased proliferation rates consistent with P values and AUC.
<i>Salmonella</i> Enteritidis	SE vs SE (C8HSL)-0.0342	SE-6.000/ SE(c8shl)-7.012	Same as above
<i>Salmonella</i> Wild Type	STWT vs STWT (C8HSL)- 0.0265	STWT-6.000/STWT(C8HSL)-7.021	Same as above
<i>Salmonella</i> Typhimurium 14028	ST14028 vs ST14028 (C8HSL)- 0.0437	ST14028-5.516/ST14028 (C8HSL)-7.017	Same as above
<i>Salmonella</i> TA 1537	STA1537 vs STA1537(C8HSL)-0.0396	ST1537-6.000/ST1537(C8HSL) - 7.034	Same as above
<i>E. coli</i>	E. coli vs E. coli (C8HSL)- 0.052	E. coli- 6.198/E. coli(C8HSL)- 6.198	C8HSL did not show any effect as p values indicate no significance consistent with AUC

3.2 Screening effects of QQ (quorum quenching) and QSM (quorum sensing) on *Salmonella* species

To investigate whether selected quorum-sensing molecules and the quorum-quenching compound farnesol altered *Salmonella* growth dynamics, *S. Typhimurium* 14028 and *S. Enteritidis* were exposed to C6HSL, C8HSL, C12HSL, and farnesol, and growth was monitored over 8 hours (Figure 3). C8HSL significantly enhanced growth in both strains, with a stronger effect observed in *S. Enteritidis*, whereas farnesol significantly reduced proliferation in both strains (Figure 3; Table 2). By contrast, C6HSL and C12HSL did not significantly alter growth profiles under the conditions tested (Figure 3; Table 2). CFU enumeration supported the absorbance-based findings, with C8HSL-treated cultures showing higher viable counts than untreated controls. Together, these data indicate that the effects of quorum-modulating compounds on *Salmonella* growth are both strain-specific and compound-specific.

383 **Figure 3. Effect of Quorum Sensing Molecules and Farnesol on the Growth Dynamics of *Salmonella***
384 ***Typhimurium* 14028 and *Salmonella* Enteritidis**



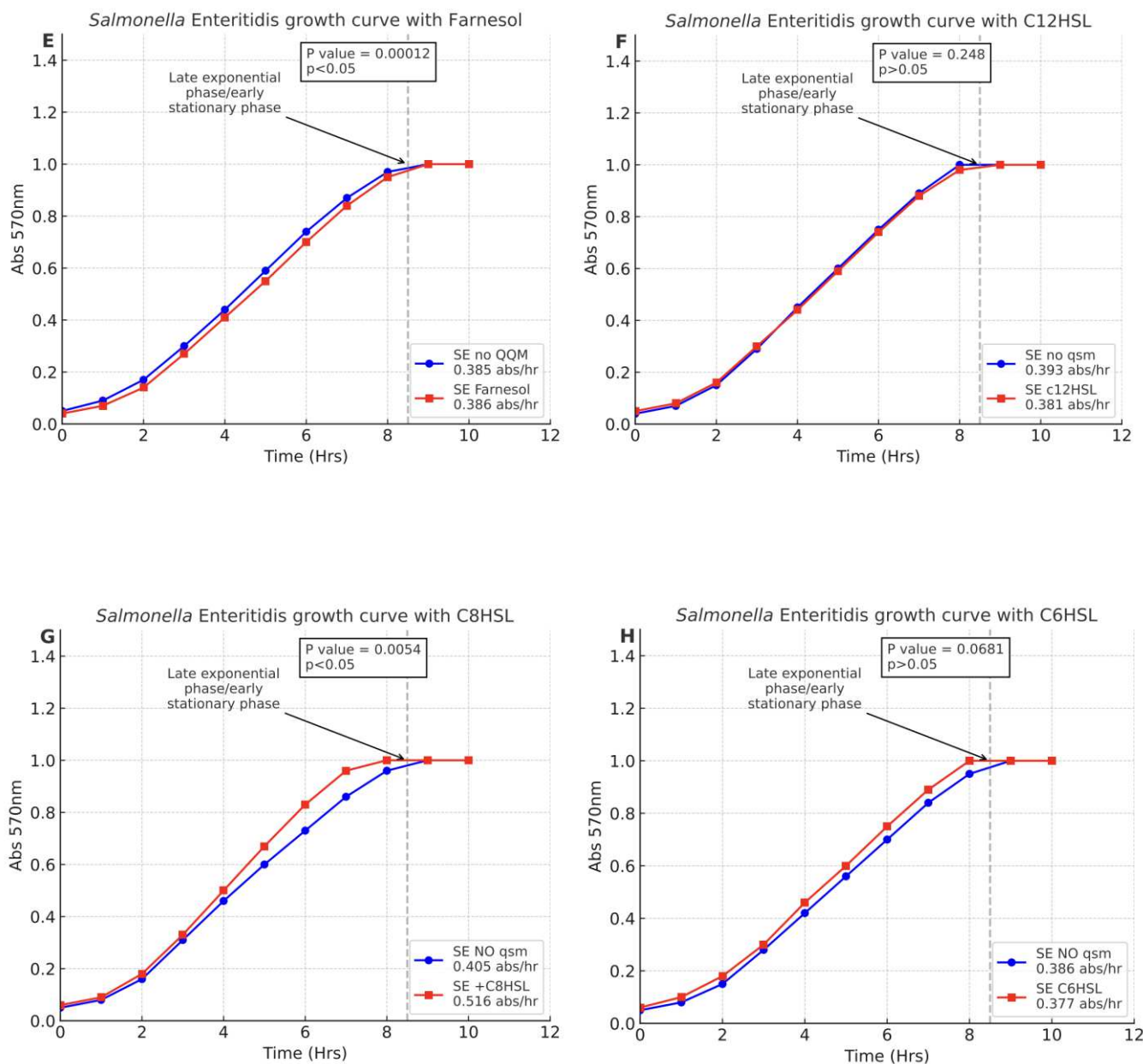


Figure 3 -Exposure to quorum sensing molecules (QSMs) and the quorum quenching compound Farnesol produced distinct effects on the growth of *Salmonella* Typhimurium 14028 (ST14028) and *Salmonella* Enteritidis (SE). C8HSL significantly enhanced growth in both strains, particularly SE, as indicated by increased growth rates and low p-values. In contrast, Farnesol significantly inhibited growth, especially in SE, despite minimal changes in final OD values. C6HSL and C12HSL had no statistically significant impact on either strain, though minor shifts in growth rates were observed. These results suggest that *Salmonella* growth responds in a strain- and compound-specific manner to QSMs and QQ agents.

CFU counts in the presence of QSM.

In support of the OD-based growth analysis, colony-forming units (CFU/mL) were also determined for each treatment condition. CFU counts for *S. Enteritidis* and *S. Typhimurium* across treatments with C6HSL, C12HSL, and farnesol remained consistent with the baseline range of 2.5×10^9 to 2.7×10^9 CFU/mL. However, in cultures

400 treated with C8HSL, CFU values increased to 3.1×10^9 CFU/mL for *S. Enteritidis* and 2.9×10^9 CFU/mL for *S.*
 401 *Typhimurium*, indicating enhanced bacterial viability and supporting the absorbance-based growth data.

402 **Growth curve result summary**

403 Growth parameters such as lag phase duration (λ), maximum growth rate ($\mu_{\max}$), and carrying capacity (K)
 404 collectively highlight the influence of quorum sensing and quorum quenching molecules on *Salmonella*
 405 proliferation. A repeated measure RM ANOVA was conducted to examine the effects of various quorum sensing
 406 molecules (C8HSL, C6HSL, C12HSL) and farnesol on the growth of *Salmonella* Enteritidis and *S. Typhimurium*
 407 strain 14028 over a 10-hour period. The analysis revealed a significant main effect of treatment and a significant
 408 interaction between treatment and time ($p < 0.05$), indicating that exposure to certain quorum-sensing molecules
 409 influences growth. Specifically, exposure to C8HSL significantly increased the growth rate in both SE ($p = 0.0054$)
 410 and ST14028 ($p = 0.0010$), enabling earlier entry into the exponential phase. C6HSL and C12HSL did not produce
 411 statistically significant changes in growth profiles for either strain. Farnesol significantly reduced proliferation in
 412 both SE ($p = 0.036$) and ST14028 ($p = 0.00012$), demonstrating its inhibitory effect on quorum-sensing-mediated
 413 growth enhancement. Post-hoc insights for pairwise comparisons also reveal the trends as indicated in table 2 below.

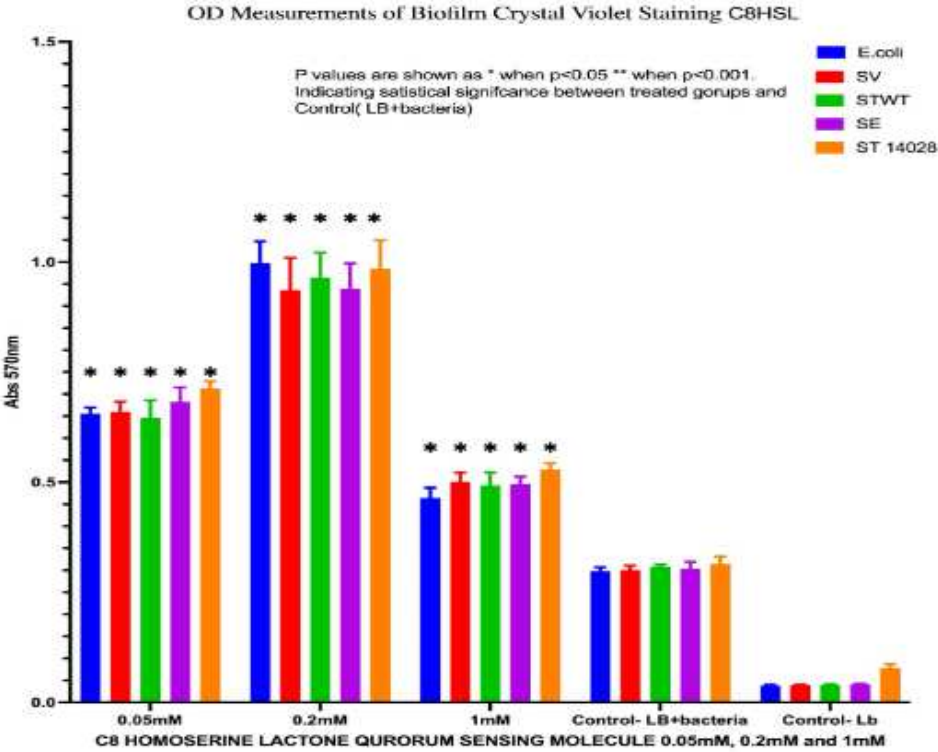
414 **Table 2- Post hoc analysis of treatment effects on the growth of *Salmonella Enteritidis* and *S. Typhimurium***
 415 **14028.**

Treatment	Species	P value	Significance	Interpretation
C8HSL Acyl homoserine lactone- medium chain length	SE	0.0054	Significant	Faster growth early exponential phase
	ST 14028	0.0010	Significant	Same effect as SE
C6HSL Acyl homoserine lactone- shorter chain length	SE	0.1000	Not significant	No clear effect on growth
	ST 14028	0.0681	Not significant	Slight trend but not statistically valid
C12HSL Acyl homoserine lactone- longer chain length	SE	0.7820	Not significant	No impact on proliferation
	ST14028	0.2480	Not significant	Same as above
Farnesol- acyclic sesquiterpene alcohol	SE	0.036	Significant	Growth rates reduced
	ST 14028	0.00012	Significant	Strong inhibition of proliferation

416

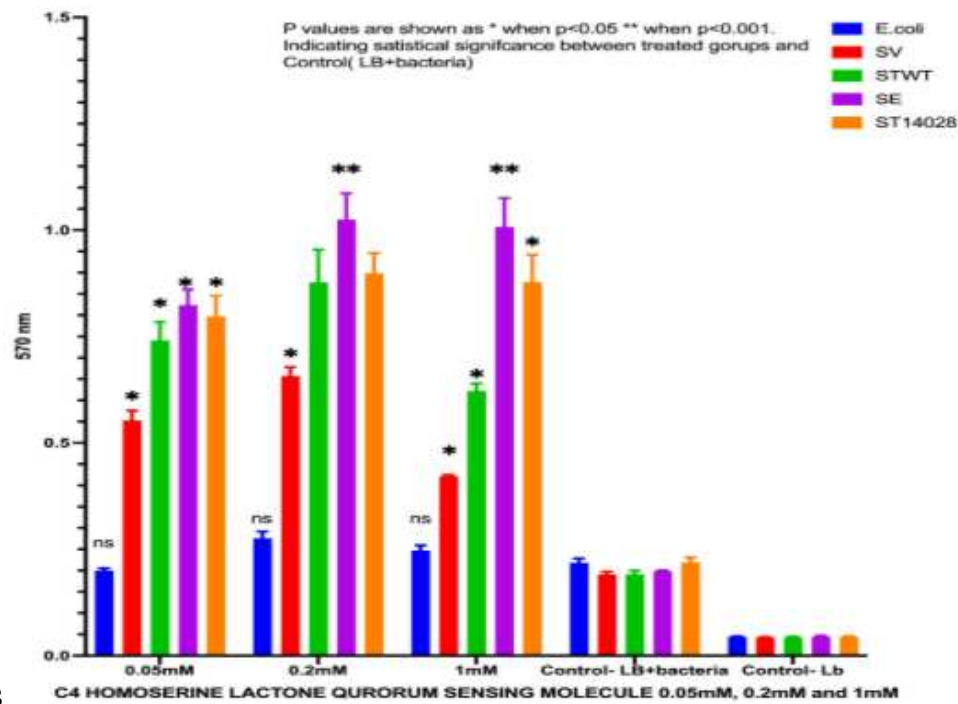
417 **3.3 Effect of quorum-sensing molecules on biofilm formation on abiotic surfaces as determined by crystal violet** 418 **assay**

419 **Figure 4. CV assay biofilm quantification in polystyrene microplate 12-well plates**
 420 Crystal violet assays were used to assess the effects of different AHLs on biofilm formation across bacterial strains
 421 grown on polystyrene surfaces (Figure 4). Among the compounds tested, C8HSL produced the strongest and most
 422 consistent enhancement of biofilm biomass across the panel, including *Salmonella* strains and, in this assay, *E. coli*
 423 (Figure 4A). C4HSL, C6HSL, and C12HSL also promoted biofilm formation, but their effects were more strain-
 424 dependent and were most evident in *Salmonella* isolates (Figure 4B–D). In several cases, higher concentrations,
 425 particularly 1 mM, were associated with reduced biofilm biomass relative to intermediate concentrations, suggesting
 426 that biofilm induction was concentration-dependent rather than linearly dose-responsive. Overall, *S. Enteritidis*
 427 exhibited the strongest response to exogenous AHLs, supporting greater sensitivity of this strain to quorum-mediated
 428 biofilm stimulation (Figure 4).

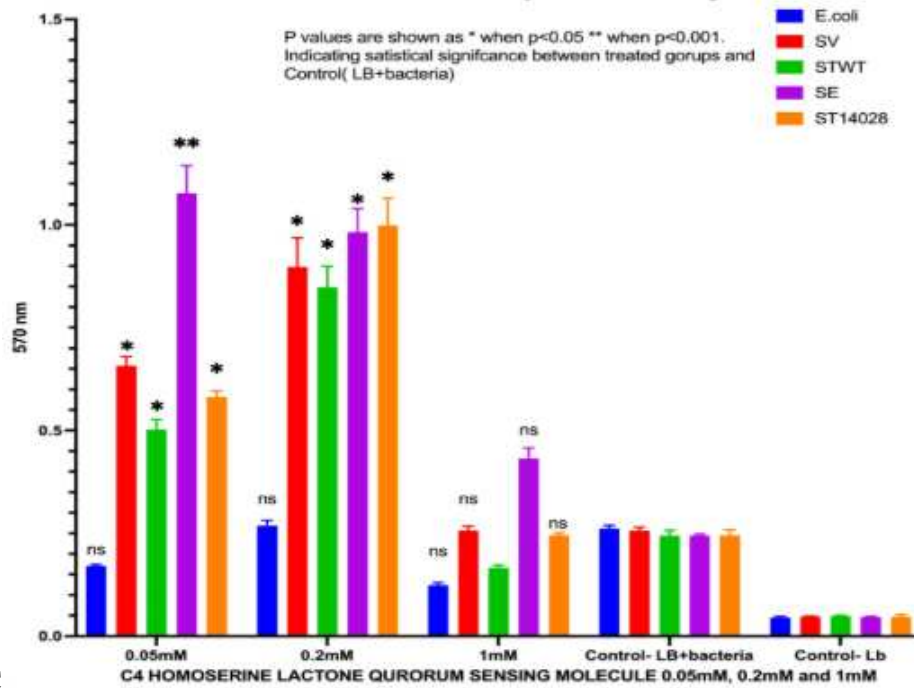


429 A

OD Measurements of Biofilm Crystal Violet Staining C4HSL



OD Measurements of Biofilm Crystal Violet Staining C6HSL



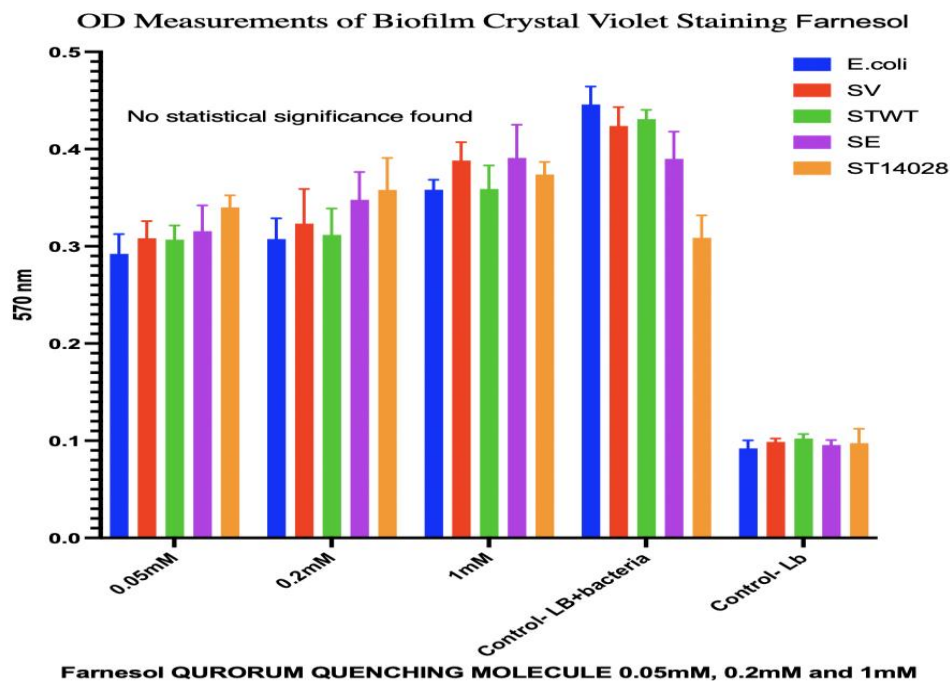
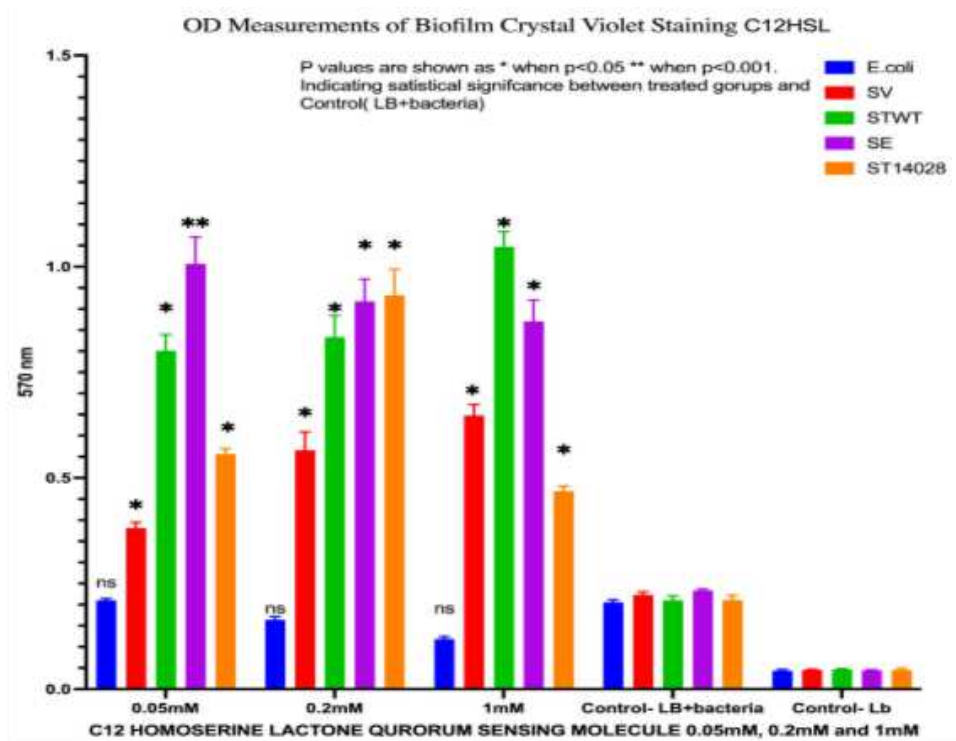


Figure 4 -Biofilm formation was significantly influenced by AHL quorum-sensing molecules, more specifically by C8HSL amongst the others listed in this study and the quorum-quenching molecule farnesol in a concentration and species-dependent manner. Treatment with C8HSL significantly enhanced biofilm biomass across all bacterial strains, including *E. coli*, at 0.05 mM, 0.2 mM, and 1 mM ($p < 0.05$). In contrast, C4HSL, C6HSL, and C12HSL significantly promoted biofilm formation primarily in *Salmonella* strains (SV, STWT, SE, and ST14028), with *E. coli* showing no significant response. *S. Enteritidis* exhibited the strongest biofilm induction, particularly under C4HSL and C6HSL treatments, with highly significant increases ($p < 0.001$). Higher concentrations (1 mM) of C6HSL and C12HSL often resulted in reduced biofilm levels, suggesting potential

inhibitory effects. Overall, quorum-sensing molecules, especially C8HSL, markedly enhanced biofilm formation, with SE showing the highest responsiveness.

3.6. Figure 5 Effect of Natural product on *S. Tyhimurium* and *S.Enteriditis* biofilm formation through CV assay.

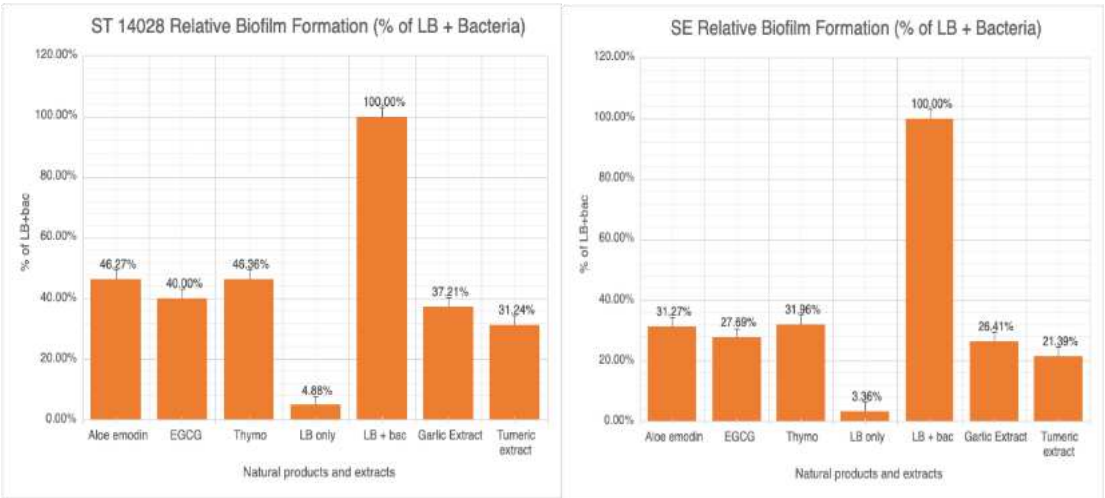


Figure 6 Effect of quorum quenchers and known inhibitors on *S. Tyhimurium* and *S.enteriditis* biofilm formation through CV assay.

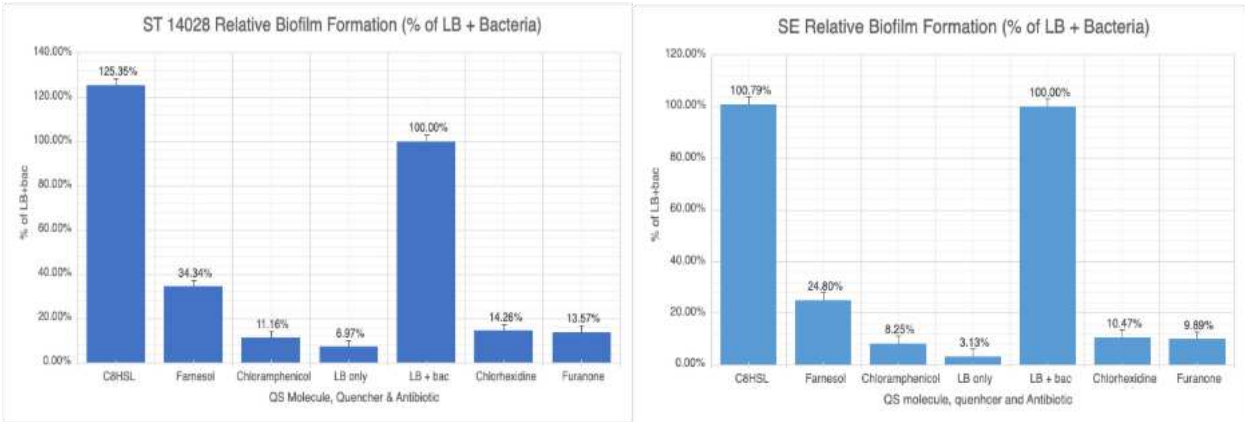


Figure 5 and 6 analysis- These CV assay graphs show how different treatments affect biofilm formation relative to the positive control (LB + bacteria = 100%).

Values below 100% indicate reduced biofilm formation, while values above 100% indicate enhanced biofilm relative to the positive control. The LB-only control (~4%) confirms minimal background staining. All tested natural

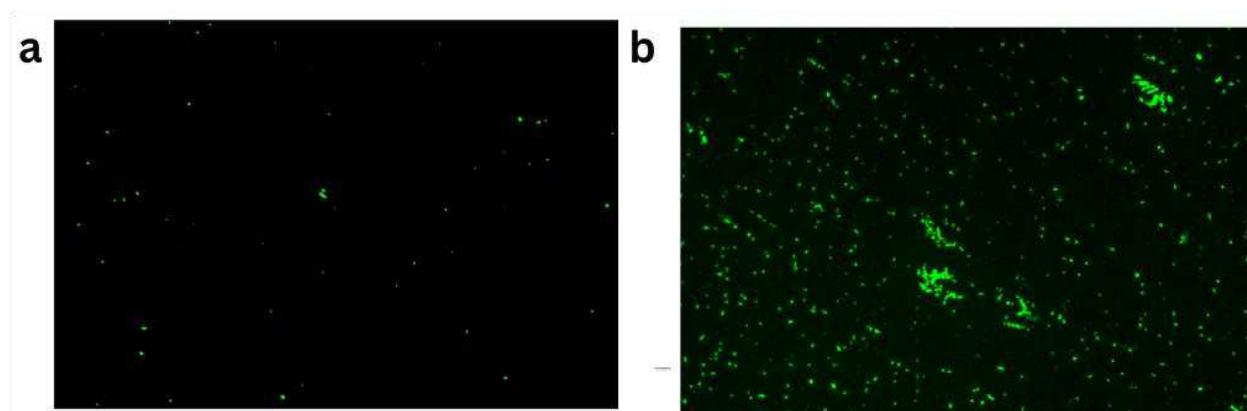
457 compounds reduced biofilm formation compared to positive control. Chlorhexidine showed the strongest effect,
458 reducing biofilm to ~15%, consistent with its known antimicrobial activity. EGCG and garlic extract demonstrated
459 strong antibiofilm effects (~26–31%), suggesting potential interference with quorum sensing or bacterial adhesion.
460 Aloe emodin and thymoquinone exhibited moderate inhibition (~40–50%), while turmeric extract also reduced biofilm
461 formation but to a lesser extent. C8HSL increased biofilm formation to ~110%, confirming its role as a quorum sensing
462 signal molecule and indicating that quorum sensing positively regulates biofilm development. In contrast, farnesol
463 reduced biofilm to ~36%, demonstrating effective quorum quenching activity.

464 Chloramphenicol reduced biofilm to ~27%, likely due to inhibition of bacterial growth and metabolic activity. the
465 consistently low LB-only value further confirms minimal background staining.

466 3.7 Fluorescence microscopy of *S. Enteritidis* biofilm formation on Caco-2 cells

467 To qualitatively assess the effect of selected quorum-sensing molecules on biofilm formation on a biotic surface,
468 confluent Caco-2 cells were co-infected with *S. Enteritidis* and exposed to C4HSL or C6HSL, followed by
469 fluorescence staining and microscopy (Figure 7). Microscopic examination indicated enhanced biofilm-associated
470 fluorescence following AHL treatment compared with the untreated control, with C6HSL producing a visibly stronger
471 effect than C4HSL (Figure 7). As this experiment was image-based, the findings should be interpreted as qualitative
472 evidence of enhanced surface-associated biofilm formation on Caco-2 cells rather than quantitative biofilm
473 measurement.

474 **Figure 7 - Effect of QSM on the *S. enteritidis* biofilm formation on biotic surfaces**

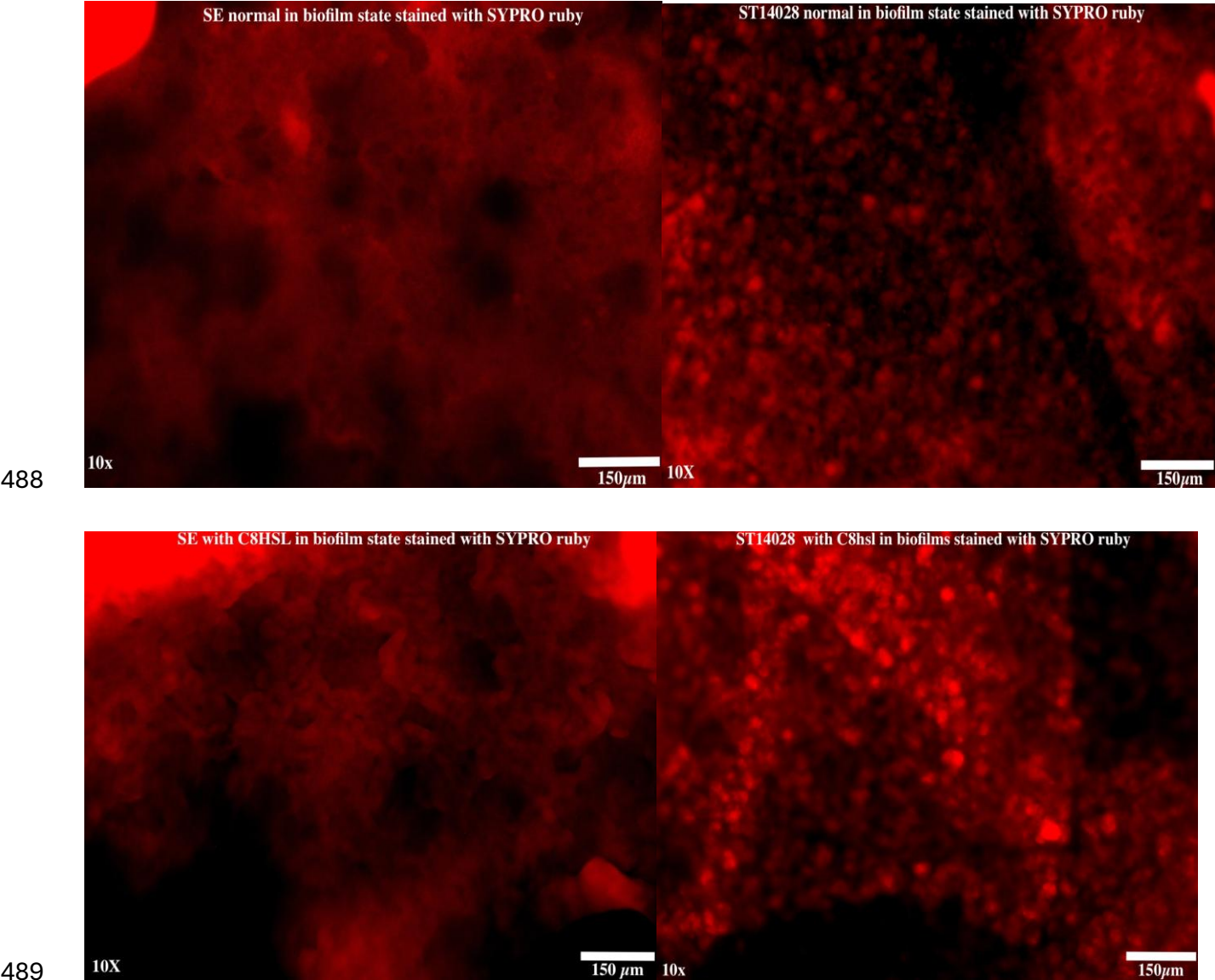


475 **Figure 7-** Caco-2 cell lines were utilised as a biotic surface model system to further investigate the effects of quorum-sensing
476 molecule (QSM) treatment. Both QSMs mentioned above were found to enhance biofilm formation on the cellular surface.
477 Notably, C6HSL exhibited a pronounced biofilm-stimulating effect at an optimal concentration of 0.5 mM. Image A represents
478 the untreated control, while Image B shows cells treated with C6HSL

480 3.8 SYPRO ruby Fluorescence microscopy

481 Biofilm architecture on glass coverslips was examined under control, C8-HSL-treated, and farnesol-treated
482 conditions using SYPRO Ruby staining and confocal microscopy (Figure 8). In both *Salmonella* strains, C8-HSL
483 treatment was associated with denser and more cohesive biofilm matrix distribution compared with untreated
484 controls, whereas farnesol treatment resulted in visibly disrupted biofilm architecture, reduced biomass, and patchier
485 surface coverage (Figure 8). These imaging data complement the CV assay findings and support the conclusion that
486 quorum sensing promotes, while quorum quenching disrupts, *Salmonella* biofilm development on abiotic surfaces.

487 **Figure 8 Effect of QSM on the Salmonella isolates biofilm formation on abiotic surface.**



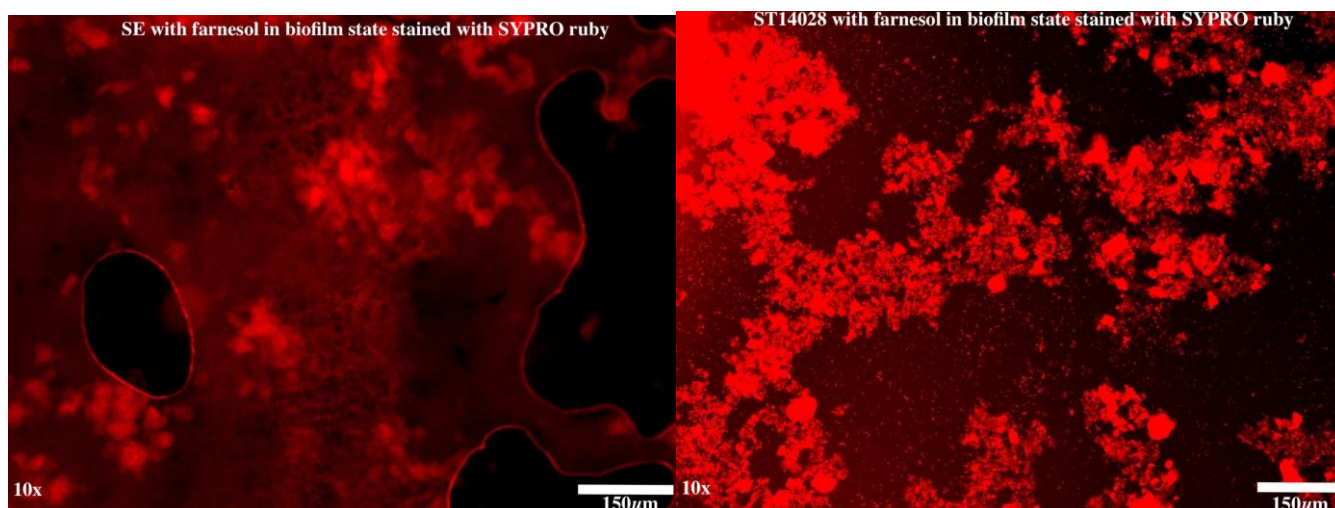


Figure 8 - In both strains, treatment with C8-HSL appeared to enhance biofilm formation, with visibly denser and more cohesive matrix distribution compared to untreated controls. In contrast, treatment with farnesol resulted in disrupted biofilm architecture, showing reduced biomass and more irregular or patchy coverage of the surface. These images illustrate the differential effects of quorum modulation on biofilm development and support the role of quorum sensing in regulating extracellular matrix production in *Salmonella*. Scale bars represent 150 µm.

Discussion

In the present study, we investigated how exogenous quorum-sensing molecules influence *Salmonella* growth and biofilm formation, and whether quorum-quenching or natural-product-based interventions can modulate these phenotypes.

Foodborne pathogens such as *Salmonella enterica* remain difficult to eradicate due to their capacity to form biofilms on both food-contact materials and environmental surfaces (Jabłońska-Trypuć et al., 2022; Pradhan et al., 2023). Biofilm formation enhances persistence and tolerance to antimicrobials and environmental stressors, increasing the likelihood of contamination, chronic colonization, and recurrent infection (Roasto et al., 2023; Liu et al., 2024).

The data indicate that quorum-sensing responses are both strain-dependent and molecule-specific. In the multi-species growth comparison, C8HSL produced a modest but consistent enhancement of early exponential growth across all *Salmonella* isolates tested, whereas *E. coli* showed no significant response (Figure 2; Table 1). This selective effect suggests that *Salmonella* can respond to exogenous AHLs in a manner that alters growth kinetics, potentially through SdiA-mediated regulation of metabolic activity or stress adaptation pathways (Sholpan et al., 2021; Zhang et al., 2022). In the focused two-strain analysis, C8HSL significantly increased growth in both *S. Enteritidis* and *S. Typhimurium* 14028, whereas farnesol reduced proliferation. The lack of significant response to C6HSL and C12HSL further indicates that AHL-mediated signaling in *Salmonella* is not uniform but instead depends on specific molecular structures and receptor interactions (Almeida et al., 2017; Campos-Galvão et al., 2015).

The abiotic biofilm assays demonstrated that exogenous AHLs enhance biofilm formation in a concentration-dependent manner, with C8HSL showing the most pronounced effect across strains (Figure 4). The heightened responsiveness of *S. Enteritidis* suggests differential sensitivity to quorum signals or variation in downstream regulatory networks controlling biofilm-associated gene expression. This is consistent with the known role of SdiA as a LuxR homolog that enables *Salmonella* to detect AHLs produced by neighboring microbial species. Through this mechanism, *Salmonella* may integrate environmental signaling cues to optimize surface attachment and biofilm

development within polymicrobial communities.

The natural-product screening provided further insight into potential biofilm control strategies. EGCG and *Allium sativum* extract demonstrated strong reductions in biofilm biomass, while *Aloe vera*, thymoquinone, and *Curcuma logan* produced moderate inhibitory effects (Figure 5). Farnesol also reduced biofilm formation, supporting its role as a quorum-quenching or biofilm-disruptive agent (Figure 6). Although precise mechanisms remain unclear, these effects may involve interference with signaling pathways, disruption of extracellular matrix production, or alterations in cell adhesion processes (Zouine, et al., 2024). As the assays used here are biomass-based, they do not distinguish between these possibilities. Nevertheless, the consistent inhibitory trends observed across multiple compounds highlight the potential of natural products as modulators of biofilm-associated phenotypes.

The Caco-2 model provided preliminary insight into host-associated biofilm behavior. Fluorescence imaging indicated increased *S. Enteritidis* biofilm-associated signal following AHL exposure, with C6HSL producing a stronger apparent effect than C4HSL (Figure 7). These observations suggest that quorum sensing may influence bacterial interactions with epithelial surfaces, potentially affecting colonisation dynamics. This was similar to studies conducted by Laura et al 2024, who concluded from their investigation that the bovine lactoferrin and chimera lactoferrin have the potential to inhibit and destroy *S. Typhimurium* biofilms. However, as this experiment was qualitative, the results should be interpreted cautiously and not as a quantitative assessment of biofilm formation on biotic surfaces.

Microscopy of biofilms formed on glass coverslips supported the findings from abiotic assays. C8HSL treated samples exhibited denser and more structurally cohesive biofilm architecture, whereas farnesol-treated samples showed disrupted organization and reduced surface coverage (Figure 8). These observations suggest that quorum sensing influences not only biofilm biomass but also structural development, potentially through regulation of extracellular polymeric substance (EPS) production and matrix organization (Zhang et al., 2022; Liu et al., 2024; Afrasiabi and Partoazar, 2024).

Several limitations warrant consideration when interpreting these findings. First, the study does not incorporate transcriptional or genetic analyses capable of elucidating the specific regulatory pathways modulated by C8-HSL or farnesol. Second, the lack of quantitative biofilm assessment within the Caco-2 model constrains the ability to draw definitive conclusions regarding biofilm dynamics in a host-associated context. Third, reliance on biomass-based assays precludes discrimination between quorum-sensing interference and nonspecific antimicrobial or metabolic effects. Accordingly, future investigations should integrate gene expression profiling, targeted mutant analyses (e.g., *sdiA*-associated strains), and viability-based methodologies to more precisely delineate the mechanisms underlying the observed phenomena.

549 Conclusion

This study highlights the significant role of interspecies quorum sensing in shaping *Salmonella enterica* behaviour, particularly in relation to biofilm development and persistence. The findings demonstrate that specific signalling molecules, notably C8HSL, can modulate bacterial growth dynamics and promote structured biofilm formation, indicating that *Salmonella* is capable of actively responding to external quorum cues within polymicrobial environments. Conversely, the observed inhibitory effects of farnesol and selected natural compounds underscore the potential of quorum-targeted strategies to interfere with biofilm establishment and stability. These results support the concept that quorum sensing represents a critical regulatory layer influencing *Salmonella* adaptation, with implications for both environmental persistence and host-associated colonization. Targeting quorum-linked pathways offers a promising alternative to conventional antimicrobial approaches by attenuating virulence and biofilm formation without directly imposing strong selective pressure for resistance. This approach is particularly relevant in food safety and clinical contexts, where biofilm-associated contamination and infection remain difficult to control. While this

561 study provides important phenotypic insights, further investigation is required to elucidate the underlying molecular
562 mechanisms. Future work should focus on integrating transcriptional profiling techniques, such as RT-qPCR and RNA
563 sequencing, alongside targeted functional analyses of key regulatory genes including *sdiA*, *csgD*, *bapA*, and *rck*. The
564 application of reversible gene-silencing strategies, such as PNA-based cell-penetrating peptides, presents a valuable
565 approach for dissecting gene function without permanent genetic modification. In addition, the development of
566 quantitative biofilm models on host-relevant surfaces, including epithelial cell systems, will be essential to better
567 reflect in vivo conditions. Expanding this work to multispecies and food-associated environments will further enhance
568 understanding of how *Salmonella* interacts within complex microbial communities. Ultimately, this research
569 contributes to the growing recognition of quorum sensing as a key determinant of bacterial behaviors and supports its
570 exploitation as a target for innovative anti-biofilm and anti-virulence strategies.

571 **Declarations**

572 **Funding:** Enhanced Funding for consumables, School of Life Sciences, University of Westminster.

573 **Data Availability Statement**

574 All relevant data generated or analysed during this study are included in the manuscript. Raw data files,
 575 including OD readings and growth curves are publicly available on
 576 <https://doi.org/10.5281/zenodo.19062812>.

577 **Ethics Statement:** The study did not involve human participants, animal models, or patient data.
 578 However, Caco-2 cell lines and *Salmonella* isolates were used following standard biosafety and handling
 579 procedures approved by the University of Westminster.

580 **Research resource Identifiers (RRID):**

581 **Salmonella enterica subsp. enterica-** RRID: NCBITaxon_59201

582 **Caco2 cell lines** – donated by Prof. Gulrez Zariwala’s lab – Centre for Nutraceuticals, School of Life
 583 Sciences, College of Liberal Arts and Sciences, University of Westminster, 115 New Cavendish Street,
 584 London, W1W 6UW. RRID: CVCL_0025

585 **Ethics Approval:** Approval was obtained from the ethics committee of University of Westminster. The
 586 procedures used in this study adhere to the tenets of the Declaration of Helsinki. Ethics Application
 587 number: [ETH2223-1279](#)

588

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592

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595 **Consent to Participate declaration:** not applicable.

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601

602 Biographical note for Schnieder Fernandes: I am a second-year PhD student conducting research on
 603 bacterial genomes with a focus on *Salmonella* serovars. My project involves using CRISPR-Cas9
 604 technology to generate mutant libraries, allowing me to investigate the functions of unknown genes. With a

605 background in cell and molecular biology as well as immunology, I am currently developing a plasmid-
606 free gene editing strategy for this research.

607 Biographical note for Anjali Ghosh: My primary research focus has been on bacterial molecular biology,
608 particularly the pathogenesis of *Salmonella enterica* serovars. During this time, I have investigated key
609 aspects of bacterial behaviour, including multidrug resistance (MDR), virulence, biofilm formation, and
610 overall pathogenesis, contributing to a deeper understanding of bacterial infections and their implications
611 for therapeutic interventions. These works have been published in peer-reviewed journals. In addition to
612 my work in bacterial molecular biology, I have also researched in immunology, focusing on ACE gene
613 polymorphism, specifically exploring their roles in conditions such as angioedema and food and venom
614 allergies. My earlier investigations into the effects of plant oestrogens on breast cancer cell lines also
615 yielded significant findings, which were published in respected scientific journals.

616 **Author Contributions:** All authors contributed equally to the study.

617 Conceptualisation: Anjali Ghosh

618 Methodology: Schnieder Fernandes, Anjali Ghosh

619 Investigation: Schnieder Fernandes, Anjali Ghosh

620 Data Analysis: Schnieder Fernandes

621 Writing – Original Draft: Anjali Ghosh

622 Writing – Review & Editing: Schneider Fernandes, Anjali Ghosh

623 Supervision: Anjali Ghosh

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