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Extracellular polymeric substances- the real target in eradicating pseudomonad biofilms

Srinithi Muthuraman *, Jon Palmer, Steve Flint

Food Microbiology- Biofilm Research Team, School of Food Technology and Natural Sciences, Massey University, 4412, Palmerston North, New Zealand

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ABSTRACT

Pseudomonads are common psychrotrophic spoilage bacteria associated with dairy, poultry, and meat processing environments. Pseudomonads can form robust biofilms at cold temperatures and produce thermostable spoilage enzymes. This review discusses the biofilm formation aspects of pseudomonads, such as biofilm formation at the air-liquid interface, psychrotrophic temperatures, and distinct EPS production. The components of the EPS produced by pseudomonads and the potential of pseudomonads as a public good provider to other bacteria within the biofilm are highlighted. The elimination strategies available, other than conventional CIP methods, were discussed. The elimination strategies either target the cells or the EPS. When the cells were removed completely, the remaining EPS footprints encouraged the robust regrowth of the biofilms and strategies targeting only the EPS, such as enzymes, led to multiple colonisation possibilities from the dispersed aggregates. Combining the cell and EPS targeting strategies would result in complete biofilm removal. However, the cost-effective production, rapid removal and safety on food matrices need to be considered while designing the control strategies of pseudomonad biofilm removal.

1. Introduction

Food spoilage by microbial contamination affects the final quality of food products, interferes with processing time and sometimes leads to financial loss (Teh et al., 2012). Pseudomonads are one such bacterial genus dominating the cold food processing conditions. In Italy, 70,000 packs of Mozzarella cheese were withdrawn from the market due to contamination and blue pigment production with *Pseudomonas fluorescens* during June 2010 (Del Olmo et al., 2018). A recent study on isolating pseudomonads from the minced and frozen meat from supermarkets revealed that around 76 % of *Pseudomonas* spp. were isolated from minced beef and 48 % from imported frozen meat in Egypt (Wehedy et al., 2025). Biofilms formed by these bacteria are a source of thermostable enzymes, pigments which affect the quality of food and provide nutrients to the cells (Teh et al., 2012). The ability to grow and form biofilms at psychrotrophic temperatures, resistance to environmental stress, thrive on simple nutrients, and adhere to both biotic and abiotic surfaces are the reasons for their persistence in the food processing environments (Sterniša et al., 2023; Ibusquiza et al., 2012). The robust EPS (Extracellular Polymeric Substances) matrix can accommodate low EPS producers and pathogens, which can be a food safety threat (Puga et al., 2018).

Pseudomonas spp. are non-sporing Gram-negative rods with flagella. *Pseudomonas* spp. are the most studied bacterial genera among Gram-negative bacteria (Silby et al., 2011). Pseudomonads fail to grow under pH 4.5 (acidic conditions), are chemoorganotrophs, oxidase-positive or negative, and catalase-positive (Lalucat et al., 2022). Some of the pseudomonads are opportunistic pathogens and phytopathogens. However, most pseudomonads are non-pathogenic (Molina et al., 2013). Pseudomonads are strict aerobic bacteria with respiratory metabolism, where oxygen is the terminal electron acceptor. However, in some pseudomonads, nitrogen can act as a terminal electron acceptor and allow it to grow under anaerobic conditions (Lalucat et al., 2022). Arginine fermentation via the arginine deiminase pathway (ADI) provides energy and glucose fermentation to ethanol via the Entner-Doudoroff pathway and contributes to long-term survival under anaerobic conditions (Kolbeck et al., 2021). Group 1 pseudomonads are psychrotrophic, fluorescent, or non-fluorescent and known for their history of food spoilage. The well-known fluorescent species are *P. fluorescens*, *P. putida*, *P. aeruginosa,* and *P. chlororaphis* (Kumar et al., 2019).

A complex enzymatic system enables good metabolic versatility

* Corresponding author.
E-mail address: s.muthuraman@massey.ac.nz (S. Muthuraman).

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among these *Pseudomonas* species (Silby et al., 2011). The optimum growth temperatures for most *Pseudomonas* species are between 25 °C and 30 °C under laboratory conditions. However, some pseudomonads can grow up to 45 °C and as low as 0 °C (Tribelli & López, 2022). Pseudomonads produce a range of proteolytic, lipolytic, and pectolytic enzymes. Proteolytic and lipolytic enzymes are often associated with the spoilage of meat, poultry, and dairy, where they break down the proteins and lipids and produce rancid odours. *P. fluorescens*, *P. viridiflava* are responsible for the spoilage of fresh fruits and vegetables due to pectolytic enzymes (Kumar et al., 2019). *Pseudomonas* spp. secretes pigments such as pyoverdine (Fluorescein), a yellow pigment, and pyocyanin, a blue pigment (Lau et al., 2004). The prevalence of *P. fluorescens* in the milk indicates the possible spoilage of milk (Ahmed & Hassan, 2024). Pseudomonads form biofilms on both biotic and abiotic surfaces. Biofilms provide a source of bacteria and enzymes contributing to spoilage in food (Machado et al., 2017). *P. aeruginosa* is considered a model organism for biofilm formation studies (McDougald et al., 2008). This review focuses on different aspects of biofilm formation of psychrotrophic pseudomonads and how biofilm footprints affect the regrowth of pseudomonad biofilms.

2. Spoilage potential of pseudomonads

The presence of the *aprX* gene is positively correlated with the proteolytic activity of spoilage pseudomonads isolated from dairy, meat, and vegetable processing sources (Caldera et al., 2016; Kumar et al., 2019). However, the proteolytic activity in this study is mainly against milk proteins. Many of the proteases from pseudomonads belong to the class of metallopeptidases (EC 3.4.24) (Caldera et al., 2016). Pseudomonads isolated from raw milk with high protease activity have a greater number of functional protease genes encoding carbohydrate transport and metabolism, signal transduction, synthesis, transport, and metabolism of secondary metabolites, polysaccharide lytic enzymes, and sugar esterase and showed higher multidrug resistance index (Du et al., 2023). Pseudomonads produce high levels of peptidases at 25 °C, followed by 10, 7, 4 and 2 °C. However, the peptidase activity is reduced from 10 to 2 °C, and storing and transporting milk between 1 and 3 °C is recommended since the proteolytic activity is lower around this temperature range (Meng et al., 2017). The dairy isolates of *P. aeruginosa* and *P. fluorescens* showed no proteolytic and lipolytic activity at 4 °C and higher activity at 20 °C. Among the *Pseudomonas* spp. two isolates showed only proteolytic activity, three showed lipolytic activity, and five showed both proteolytic and lipolytic activity (Hoda, 2012). In *P. fragi, apr D* regulates the protease secretion and inversely affects auto-aggregation, swimming motility, and biofilm formation. However, there are other enzymes involved in slime and off-flavour production during meat spoilage (Wang, Kyere, & Ahmed Sadiq, 2021). In sheep milk stored at 4 °C, a high frequency of the *aprX* gene is found in *P. putida*, *P. fluorescens,* and *P. aeruginosa,* showing the spoilage potential of these psychrotrophs at cold temperatures. Milk stored at 9 °C facilitated the lipolytic activity of *P. putida* (Bruzaroski et al., 2023). Lack of pyoverdine secretion in *P. fluorescens* leads to weak biofilm formation and low levels of EPS production, affecting its spoilage potential (Chen et al., 2023). Quorum-sensing molecules can modify the spoilage characteristics of pseudomonads. N-acyl-homoserine lactones, also known as AHLs, affect the cell growth, spoilage protease and lipase activity, and biofilm formation of *P. koreensis* PS1 by utilising C6-HSL-dependent. However, the regulatory mechanism of AHL-induced quorum sensing on biofilm formation needs to be studied (Dai et al., 2022). The gene *rpoN* regulates swimming motility, biofilm formation, resistance to antibiotics, and spoilage potential by controlling flagellar mobility, adhesion, polysaccharide metabolism, resistance and amino acid transport, and metabolism of *P. fluorescens* (Liu et al., 2021).

3. Biofilm formation of pseudomonads

Biofilm formation by pseudomonads consists of a series of sequential events. Starved cells attach to the biotic or abiotic surface, which is known as the reversible attachment (Hinsa et al., 2003). High levels of intracellular c-di-GMP facilitate the production of adhesins and the initial attachment (Fazli et al., 2014). This stage of biofilm formation is usually considered weak and can be easily disrupted (Hinsa et al., 2003). When cells attach to the long axis and form a monolayer of cells known as irreversible attachment (O'Toole et al., 2000; Hinsa et al., 2003). Microcolonies will be formed from the monolayer of cells and develop into mature biofilms. Finally, the low levels of cyclic c-di-GMP downregulate the production of adhesins and extracellular polymeric substances and lead to dispersion (Fazli et al., 2014).

The timeline to reach the 5 stages of biofilm formation varies among the species, and this variation is even observed across strains. A study based on biofilm formation of isolates from the meat industry showed that the initial attachment of *P. lundensis* happened after 2 h of post-inoculation, and most of the population adhered after 4 h of inoculation. After initial attachment, the cells started producing extracellular matrix substances, which produced notable biofilm structures (Liu et al., 2015). The biofilm formation of *P. putida* increases after 4–6 h of incubation, and this gradually decreases when the biofilm attains maturity, and the hydrophilic surface favours its initial adhesion (Puhm et al., 2022). Some studies reported that *P. putida* forms biofilms on hydrophobic surfaces, as it is considered a stress response mechanism. To date, it is contradictory which surface facilitates adhesion in which mechanism (Ueda et al., 2015; Liu et al., 2021).

Biofilm formation is advantageous for pseudomonads. Biofilm EPS acts as a nutrient reservoir, protects the cells from desiccation and resists other antimicrobials. *P. fluorescens* C224 planktonic cells showed proteolytic activity at 20 and 30 °C, and no growth at 37 °C, while the biofilm cells grown on a stainless-steel surface showed proteolytic activity at 20, 30 and 37 °C. Biofilm formation facilitated the growth of this pseudomonad at an adverse temperature (Teh et al., 2012). Biofilm EPS acts as a nutrient reservoir, protects the cells from desiccation and resists other antimicrobials (Mann & Wozniak, 2012).

4. Biofilm formation of the pseudomonads at the air-liquid interface

Surfaces under different interfaces affect the biofilm formation including initial attachment, biofilm maturation, detachment, and interaction with environmental factors (Ye et al., 2022). Flagella play an important role in the air-liquid interface biofilm formation of pseudomonads. The thick air-liquid interface biofilm formation appeared from 48 to 144 h (Sung et al., 2024). Proteins involved in flagellar structure, type I and IV secretion systems, alginate/siderophore synthesis, quorum sensing, and c-di-GMP signalling exhibited significant upregulation between 48 and 72 h in the air-liquid interface biofilms of *P. aeruginosa* (Sung et al., 2024). The diguanylate cyclase encoding pathways known as wrinkly spreaders (Wps), the air-water surface (Aws), and the micro-water-surface (Mws) are known to mediate the cellulose production and air-liquid interface biofilm formation in *P. fluorescens* SBW25 (Ardré et al., 2019). Biofilms of *P. aeruginosa* grown under liquid-liquid conditions are heterogeneous and more susceptible to antimicrobials. However, pseudomonad biofilms formed at the air-liquid interface can be a hundred times more resistant than liquid-liquid biofilms due to the EPS overproduction acting as a physical barrier, and the cell density at the air-liquid interface is higher than the liquid-liquid interface biofilms (Fig. 1) (Tan et al., 2024). Air-liquid interface biofilms provide the bacteria with oxygen and nutrients, while the liquid-liquid interface provides nutrients and limited oxygen (Fig. 2) (Ye et al., 2022). Most of the biofilm formation models, such as microtiter plates, CDC (Centre for Disease Control) biofilm reactors, Calgary devices, and rotating biofilm reactors, often focus on biofilm formation at

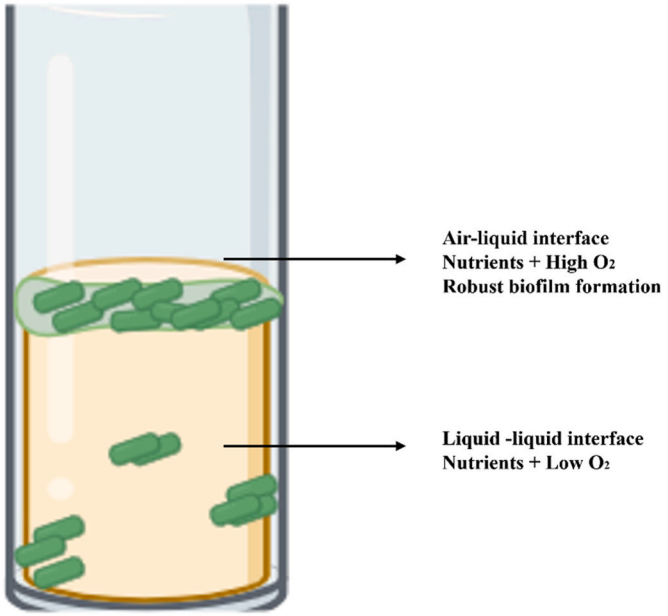

Fig. 1. Biofilm formation of pseudomonads at the air-liquid and liquid-liquid interfaces (Created in https://BioRender.com).

the solid-liquid or liquid-liquid interface. However, air-liquid biofilm formation influences attachment, nutrient uptake, and mass exchange (Zhang et al., 2022). When the clean-in-place (CIP) of air-liquid and liquid-liquid biofilms of *P. aeruginosa* PAO1 revealed that the air-liquid interface biofilms require twice the Minimal biofilm eradication concentration MBEC of CIP than the liquid-liquid biofilms (Ye et al., 2022). The spoilt-meat-associated *Pseudomonas* show 88 % of the isolates formed biofilms at the air-liquid interface, indicating the deep-rooted ability within the genus (Robertson et al., 2013). The physical disruption of these air-liquid interface biofilms resulted in fragments, flocs, and slimes. Compared to individual bacteria, these aggregates will be more effective in new colonisation after dispersion (Robertson et al., 2013). The air-liquid interface is often seen in food processing environments such as partly filled tanks, storage silos, and residual liquid after cleaning. Biofilm formation at the air-liquid interface leads to serious contamination and resistance to cleaning (Jha et al., 2020).

5. Robust biofilm formation at psychrotrophic temperatures

Cold temperatures in food processing are usually considered to limit microbial activity. However, pseudomonads can form robust biofilms in cold processing conditions (Liu et al., 2023). The robust biofilm formation at low temperatures is achieved by reducing the growth rate and matrix overproduction. The psychrotrophic pseudomonad biofilms grown at 25 °C and 4 °C reached similar cell counts in the matured phase, and at 4 °C, the overproduction of exopolysaccharides and proteins was observed (Wickramasinghe et al., 2020). The stress created by low temperatures can stimulate the EPS production of these psychrotrophic pseudomonads. The matrix overproduction is achieved by upregulating the exopolysaccharides-producing genes such as *algK* and *pslA* at 4 °C (Liu et al., 2023). Reducing motility at cold temperatures is eventually caused by the downregulation of flagellar genes and encourages the switch from planktonic cells to biofilms (Fig. 3). At 4 °C, the swimming motility was completely repressed, and higher biofilm formation was observed (Guttenplan & Kearns, 2013). Though the metabolic rate is lowered at refrigerated temperatures, the bacteria can survive, multiply, and form uniform layers of biofilms. When the pseudomonads were allowed to form biofilms at 4 and 10 °C, the planktonic cells were completely absent after 7 days for 4 °C grown biofilms and 3 days for 10°C-grown biofilms. This indicates the absence of planktonic cells in the mature biofilms (Wickramasinghe, Ravensdale, Coorey, Dykes, & Scott Chandry, 2019). Another important molecule in biofilm formation is cyclic di-GMP, which can suppress flagella-mediated swimming and promote matrix overproduction (McDougald et al., 2012). Genes regulating the biosynthesis of alginate, cellulose, and colonic acid are highly expressed in *P. fragi* biofilms grown in meat processing conditions at 10 °C (Wagner et al., 2021). Psychrotrophic pseudomonads overcome cold stress by different mechanisms such as over-expression of Cap, Csp proteins, cell membrane adaptations, dense and rigid biofilm formation, down-regulation of flagellar motility, overexpression of antioxidant enzymes, no mitochondrial swelling, accumulation of osmotic solutes and amino acids and, upregulation of cryoprotective amines (Chauhan et al., 2013). The cell division, cell cycle and chromosome partitioning genes are downregulated more than upregulated in different stages of biofilm formation, such as initiation, maturation and dispersion (Wickramasinghe et al., 2021). The highest percentage of upregulated genes belongs to carbohydrate transport, metabolism and ribosome modulation factor (RMF). Upregulation of RMF is essential to maintain the structure and cell viability under cold temperatures (Wickramasinghe et al., 2021).

6. EPS produced by pseudomonads

Pseudomonad biofilm EPS is composed of insoluble, soluble, and capsular polysaccharides, proteins, and eDNA, and the matrix overproduction is observed at the psychrotrophic temperatures (Table .1) (Liu et al., 2023; Muthuraman et al., 2025; Wickramasinghe et al., 2020). Psl, Pel, alginates, and cellulose are the common polysaccharides found in pseudomonad biofilms (Chung et al., 2023). Adhesins, curli fibres, polysaccharide binding proteins, eDNA binding proteins, and flagellin are the proteins found in the matrix of pseudomonad biofilms. Pseudomonads also produce lectins and extracellular enzymes such as arginine deiminase in their biofilms (Flemming & Wingender, 2010). The functional amyloid-like fibres in pseudomonads are termed curli fibres. The strain *Pseudomonas fluorescens* UK4 exhibits curli production and imparts robust biofilm formation (Zeng et al., 2015). In densely

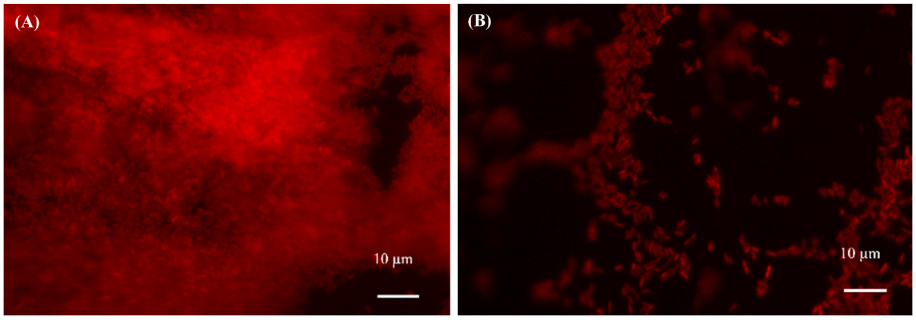

Fig. 2. The biofilm architecture between the (A) air-liquid interface and (B) submerged biofilms (scale bar 10 μm).

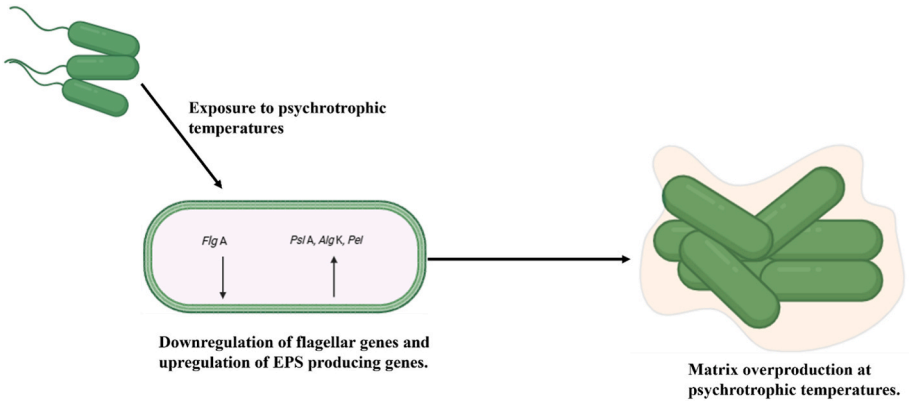

Fig. 3. Biofilm formation of pseudomonads at psychrotrophic temperatures by reducing motility and matrix overproduction (Created in https://BioRender.com).

packed biofilms of *P. fragi* and *Vibrio cholera*, a protein called RbmA is isolated, which might have a role in keeping molecules together (Drescher K et al., 2016). Extracellular DNA, also known as eDNA, can be actively secreted by the bacteria or formed from the dead biofilm cells involved in biofilm functions. However, the secretion mechanisms of eDNA still need to be explored (Flemming & Wingender, 2010; Wickramasinghe et al., 2020) (see Table 2).

6.1. Distinct polysaccharides and proteins in the EPS matrix of pseudomonads

Pseudomonads are known to produce more polysaccharides in their EPS matrix than produced in planktonic cells. A study comparing the biofilm EPS composition of *B. subtilis* and *P. aeruginosa* showed that the biofilm EPS produced by *Bacillus* contains proteinaceous substances when observed with FTIR (Fourier Transform Infrared Spectroscopy), while the pseudomonad biofilms showed signals for lipopolysaccharides, alginate, Pel and Psl. The presence of polysaccharides in the EPS matrix promoted more adhesion strength of the EPS (Harimawan & Ting, 2016). The Pel and Psl polysaccharides are responsible for the biofilm architecture of pseudomonads. Deletion of the Psl regulating gene in *P. aeruginosa* resulted in no macrocolony (mushroom-shaped structures with both motile and non-motile cells) formation, and deletion of both Pel and Psl producing genes resulted in the absence of both micro and macrocolony formation (Yang, Hu, et al., 2011). The absence of micro and macro colonies resulted in differences in antimicrobial resistance (Yang, Liu, et al., 2011). Another distinct polysaccharide produced by mucoid pseudomonads is alginate, and the interaction of alginate with divalent cations can result in the formation of a gel. The increase in alginate and Pel content will increase the yield strain of the biofilms under mechanical stress (Di Martino, 2018). The Psl, Pel, and alginate were absent in the *S. aureus* and *L. monocytogenes* biofilms; instead, the EPS matrix of these bacteria consists of lipoteichoic acid *(LTA*) and poly-N-acetyl glucosamine (PNAG) (Colagiorgi et al., 2016). The differences in their matrix composition are reflected in the biomass. Monospecies biofilms of *P. fluorescens* produced 2-fold higher biomass than monospecies *L. monocytogenes* biofilms (Puga et al., 2018).

The protein CdrA in the biofilms can bind cells to Psl and cause aggregation and biofilm stability. In the absence of Psl, it can bind with Pel and promote biofilm formation (Reichhardt et al., 2020). The CdrA and Psl are present in the biofilm matrix of pseudomonads, consisting of robust, tightly packed aggregates that keep the matrix protease resistant (Reichhardt et al., 2018). While the other bacteria possess different proteins and polysaccharides in their matrix, pseudomonads possess Cdr A, type IV pili and lectins (Lec A/Lec B) (Karygianni et al., 2020).

Compared with the two-day-old biofilms of *S. aureus, P. aeruginosa* PAO1 forms flat, tightly packed biofilms with more coverage and exhibits resistance to phagocytosis (Yang, Hu, et al., 2011). All three strains of *S. aureus* tested formed loosely packed, irregular microcolony structures with less surface coverage than *P. aeruginosa* PAO1. The binding of eDNA and type IV pili in the pseudomonads supported the co-culture of *S. aureus* and improved the density (Yang, Hu, et al., 2011). When comparing the EPS production between *P. fluorescens* PF2 and *S. Typhimurium* N25, *P. fluorescens* produced more polysaccharides and proteins on both stainless-steel and polystyrene surfaces (Yuan et al., 2025). Compared to other bacteria, *Pseudomonas* Spp. possess distinct polysaccharides in the EPS matrix, which strengthen the biofilm.

6.2. Interaction between EPS components

The different EPS components balance each other and keep the integrity of the biofilms. In *P. putida*, the mutants lacking major adhesin genes *LapA* and *LapF* resulted in overproduction of EPS (Martínez-Gil et al., 2013). In *P. aeruginosa*, the absence of Psl polysaccharide induces the overexpression of Pel, and the absence of Pel induces the overproduction of alginate, which protects the cells from adverse conditions (Ghafoor et al., 2013). The interaction of Psl and Pel is responsible for microcolony formation, and the type IV pili and eDNA interaction is essential for less heterogeneous, tightly packed flat biofilms (Yang, Hu, et al., 2011). The eDNA and Psl interaction results in a thick rope-like structure, which is essential for resisting enzymatic dispersion and provides framework to the biofilms in *P. aeruginosa* (Wang et al., 2015).

Differences in the EPS composition were observed even between the different species of pseudomonads. The monosaccharides present in the *P. aeruginosa* are mannose and mannitol, while in *P. putida*, galactose and glucose were present when forming biofilms on xylose-based growth medium (Celik et al., 2008). In mucoid strains, alginate is overexpressed, and in the non-mucoid pseudomonads, Psl takes over the role of alginate (Di Martino, 2018). Levan is a unique polysaccharide produced by soil pseudomonads. Levan acts as a nutrient reservoir in the biofilms and was seen to fill the voids of the biofilms and not participate in the biofilm architecture (Laue et al., 2006; Mann & Wozniak, 2012). When the biofilms of *P. lundensis* and *P. fragi* were grown at 25 and 10 °C, the EPS production was higher at 10 °C, and *P. lundensis* produced significantly higher proteins in the EPS matrix, while *P. fragi* produced higher polysaccharides (Wickramasinghe et al., 2020). The polysaccharide proportion in the EPS was higher in *P. fluorescens* (PF07) biofilms compared to *P. lundensis* (PL28) and *P. psychrophile* (PP26) when grown at 4 °C. This difference is reflected in the biomass residue after treatment with sodium hypochlorite. The residues left by PF07 (93.76 %) were higher than PL28 (73.29 %) and PP26 (85.65 %), but the protein concentrations in the untreated EPS were similar (Liu et al., 2023).

6.3. Factors affecting the EPS production

Temperature, pH and the presence of cations can affect the biofilm

Table 1
Components of EPS present in pseudomonad biofilms.

Components	Role	Isolate	Reference
Polysaccharides			
Alginate	Structural stability, Water and Nutrient Retention	*Pseudomonas aeruginosa*, Mucoid variants of *Pseudomonas fluorescens* *P. putida and P. syringae*	Mann and Wozniak (2012). Chung et al. (2023).
Levan	Nutrient reservoir	*P. syringae*	Laue et al. (2006).
Polysaccharide synthesis locus (Psl)	Biofilm architecture,	*P. aeruginosa*	Starkey et al. (2009).
	Protection against neutrophils and immune effectors	*P. syringae*	Winsor et al. (2009).
		P. medocina *P. fluorescens*	Chung et al. (2023)
Pel	Pellicle formation.	*P. aeruginosa*	Jennings et al. (2015)
	Compensate for the role of *Psl* during its absence. Aggregation in the broth culture.	*P. fluorescens* Pf5	Le Mauff et al. (2022)
Cellulose	Air-liquid interface biofilms.	*P. aeruginosa*	Winsor et al. (2009)
		P. lundensis	Ardré et al. (2019)
		P. cedrina	Muthuraman et al. (2025)
		P. fluorescens	
Proteins			
CdrA	Interact with the Psl and Pel polysaccharide	*P. aeruginosa*	Reichhardt (2023).
Lap A and Lap D	Cell interconnection	*P. aeruginosa*	Gjermansen et al., 2010.
	Surface adhesion	*P. fluorescens* *P. putida* *P. syringae*	
Cell appendages	Flagella and type IV pili. Forms mushroom-like structures	Most of the Pseudomonads	Patel and Gajjar (2022)
Functional amyloid-like proteins	Initial adhesion and aggregation	*P. fluorescens*	Dueholm et al., 2010.
		P. aeruginosa	
Lipids			
Rhamnolipids	Biofilm remodelling and Dispersion	Most of the pseudomonads	Abdel-Mawgoud, Lépine, & Déziel, 2010.
eDNA	Intracellular connector	Most of the pseudomonads	Muthuraman et al. (2025)
	Nutrient reservoir		Dai, Luo, et al., 2024,
	Mediate biofilms in the absence of polysaccharides		Flemming and Wingender (2010).

formation of pseudomonads. A study comparing the biofilm formation of *P. fluorescens* and *L. monocytogenes* under cold and acidic conditions found, *P. fluorescens* produced higher amounts of proteins and polysaccharides when grown at 4 °C (both pH 5.4 and 7.0), which is reflected in the crystal violet biofilm test values. This study suggests that cold and acidic stress together facilitate strong biofilm formation of *P. fluorescens* (Zhou, Dong, et al., 2024). A mild acidic pH (5.0) environment encouraged thicker biofilm formation and overexpression of rhamnolipid and alginate producing genes, which resulted in increased biomass production compared to the neutral (7.0) pH environment in *P. aeruginosa* biofilms (Mozaheb et al., 2023). When comparing the EPS production by *P. aeruginosa*, *Micrococcus* sp. and *Ochrobacterium* spp. at three different pH levels (7.0, 8.0, 9.0), *P. aeruginosa* (>300 mg/mL) EPS production was not affected and higher than the other two bacteria (<300 mg/mL) at all the pH levels. Cations are present in all the food processing environments. For example, the presence of Ca^{2+} in dairy processing, and the presence of Na and Mg^{2+} in seafood processing. Supplementation of 5 and 10 mM Ca^{2+} ions resulted in higher polysaccharide production in the EPS and complex biofilm structures with more cell clusters in *P. fluorescens* PF4 (Yuan et al., 2024). The robust biofilm formation on meat and in dairy processing environments indicates that pseudomonads can thrive in protein-rich environments together with ions (Wickramasinghe, Ravensdale, Coorey, Chandry, & Dykes, 2019).

7. Pseudomonads produce public goods

Public goods are metabolically expensive products secreted by one bacterium and utilised by other bacteria in the same community without being involved in production. Siderophores, exoproteases, and cyclic lipopeptides are the molecules produced by *Pseudomonas* spp. as public goods (Loarca et al., 2019; O'Brien et al., 2017). Bacterial volatile compounds from *P. fluorescens* act as a public good and interact with *L. monocytogenes* across the physical barriers, promote motility, and encourage biofilm formation (Zhou, Liu, et al., 2024). Diffusion of public goods in planktonic form is much more effective than diffusing in tightly packed, spatially structured biofilms. In *P. aeruginosa* biofilms, maximum concentrations of pyoverdine are observed at the centre of the biofilms and this local trafficking of public goods modulates the growth of the community. However, the molecular mechanisms that regulate the public goods distribution need to be addressed (Julou et al., 2013). When specialization is enforced in the production of public goods (siderophores) results in mutual cheating rather than efficient division of labour in *P. aeruginosa* under iron-limiting conditions (Mridha & Kümmerli, 2022). Rhamnolipid is a biosurfactant produced by pseudomonads that can be exploited by other bacteria for swarming motility, mediate assimilations of hydrocarbons as nutrients, and change the biofilm architecture. However, *P. aeruginosa* controls the rhamnolipid production based on the growth rate rather than cell density, which regulates the rhamnolipid biosynthesis genes (Guadarrama-Orozco et al., 2023). Surfactants achieve phyllo spore colonisation by pseudomonads during fluctuating humidity. The surfactant produced by *Pseudomonas* spp. FF1 encouraged the co-swarming of *Pantoea eucalypti* 299 R. *P. eucalypti* biomass was significantly increased compared to its monoculture or co-swarming with biosurfactant mutant pseudomonads (Kunzler et al., 2024). EPS, eDNA, and some biofilm proteins can be exploitable as public goods as they enable protection from adverse conditions, and aid in the adhesion and attachment process (Guadarrama-Orozco et al., 2023). *L. monocytogenes* is a "cheater" when co-cultured with *P. fluorescens* as its a poor EPS producer and shelters under pre-formed pseudomonad biofilm, and this highlights the sharing of EPS as a public good (Puga et al., 2018). Scanning electron microscopy of *L. monocytogenes* and *P. aeruginosa* cultures showed that after 24 h the former was still in its adhesion stage while *P. aeruginosa* formed denser biofilms and the structure of the co-culture biofilms is the same as the *P. aeruginosa* biofilms, which reveal the dominance of *Pseudomonas* spp. in the EPS production (Dong et al., 2022). The advantages of this EPS for *Listeria* are providing nutrients, protection from external stress, reduced diffusivity of chemicals, and prolonged survival. This cooperative behaviour depends on the species and their concentration (Dong et al., 2022).

8. Biofilm-related genes in pseudomonads

A ubiquitous second messenger in proteobacteria is known as cyclic dimeric GMP (c-di-GMP), which upregulates biofilm formation (Fig. 4). Putrescine and arginine enhance the biofilm formation of *P. aeruginosa* PAO1 with increased levels of c-di-GMP (Liu et al., 2022). Flagella (*flgA*), quorum sensing (*luxR*), exopolysaccharides (*algK, pslA*), and stress response (*rpoS*) are found in *P. fluorescens* PF07, *P. lundensis* PL28, and *P. psychrophile* PP26, and their relative expression is higher at 4 °C compared to 25 °C (Liu et al., 2023). The gene *aprD* is known for

Table 2
Comparison of the biofilm elimination strategies of pseudomonads.

Biofilm eradication strategies	Target	Cost effectiveness	Efficacy	Challenges	References
Traditional CIP involves the use of hot water, alkali, and acid treatments.	Cells	Low	Industry standard Remove food debris Remove minerals	High energy and water use Salt residues EPS remnants Corrosion of metal by acid treatment Crew and crevices can accommodate biofilms	Parkar et al. (2004) Simões et al., (2010) Pant et al. (2023)
Chlorination	Cells	High	High oxidation capacity (Chlorine dioxide)	Chlorate deposition and health risk Impact on food matrix Corrosion Chlorate resistance	Pant et al. (2023) Gagnon et al. (2005)
Enzyme cleaners	EPS	Low (Require a combination of enzymes or surfactants, or other molecules that increase the production cost).	Disperse the biofilms Environment friendly Biodegradable	Possible recolonisation The interaction between the matrix components can keep the matrix resistant to enzymes Residual enzymes can react with food matrices.	Nahar et al. (2018) Eladawy et al. (2020) Baker et al. (2016) Rubio-Canalejas et al. (2022)
Biosurfactants	Cells	Low (High production cost)	Prevent the cell attachment and biofilm formation Biodegradable Prevent corrosion	Cannot eliminate mature biofilms. Only a few, such as sophorolipids, possess bactericidal activities.	Bhadra et al. (2023) Jimoh et al. (2023) Díaz De Rienzo et al. (2015)
Ultrasound	Cells	Low (High operational cost and treatment time)	Prevents bacterial adhesion Mechanical disruption of the EPS matrix	Stimulated alginate production in *P. aeruginosa* due to mechanical stress. The effects on food quality are not well known	Yu et al. (2020) Erriu et al. (2014) Lambert et al. (2010)
Quorum-quenching molecules	Cells	Low (Longer treatment time)	Reduction in bacterial adhesion Inhibit microcolony formation Biodegradable	Possibilities of developing resistance towards quorum-quenching molecules. Not a universal solution	Paluch et al. (2020) Krzyżek (2019)
Sanitizers	Cells	Moderate to high (However, the biodegradable sanitizers require high concentration).	Prevents biofilm formation. Targets (peracetic acid and hydrogen peroxide) include a broad spectrum of bacteria.	Reduced diffusivity due to EPS Not all sanitizers are environmentally friendly Risk of biocide-induced antimicrobial resistance. Some sanitizers are corrosive, unstable, and explosive.	Fernandes et al. (2024) Jones and Joshi (2021) Dawan et al. (2025) Chowdhury et al. (2025)
Photodynamic inactivation	Cells	Low (Scaling up needs to be considered from an industrial point of view)	Rapid microbial killing. The reactive oxygen species can target cells and some molecules (polysaccharides are susceptible to photodamage) in the EPS. No effects on Food matrices.	Reactive oxygen species can affect the food quality. Minimal effects on the EPS matrix. Require combined treatment to eradicate the biofilms. Limited penetration depth.	Yuan et al. (2021) Wang, Qing, Tang, et al. (2021) Cieplik et al. (2014) De Melo et al., 2013
Cold atmospheric plasma	Cells	Low (Requires double the cost compared to chlorination, as it relies completely on electricity).	Preserving bioactive components in food matrices. No added chemicals. Minimal adverse effects on the organoleptic properties of food.	Reactive species can break down the lipid bonds and have an impact on food quality	Zhu et al. (2020) Naicker et al. (2023)

controlling the secretion of proteases in Pseudomonads. However, the deletion of *aprD* genes leads to a disorganized biofilm surface and changes in EPS matrix components such as proteins and polysaccharides. The deletion of *aprD* increased the motility of *P. fragi* and produced sparsely distributed cell aggregates (Wu et al., 2022). The functional amyloid (Fap) in *P. fluorescens* PF07 is required for the formation of microcolonies, pellicles, and solid surface-associated biofilms. The bacterial enhancer-binding protein *brfA* regulates the fap-dependant biofilm formation by sensing c-di-GMP (Guo et al., 2024). The gene *rpoN* and *rpoN-dependent* promoters regulate the *fapABCDE*

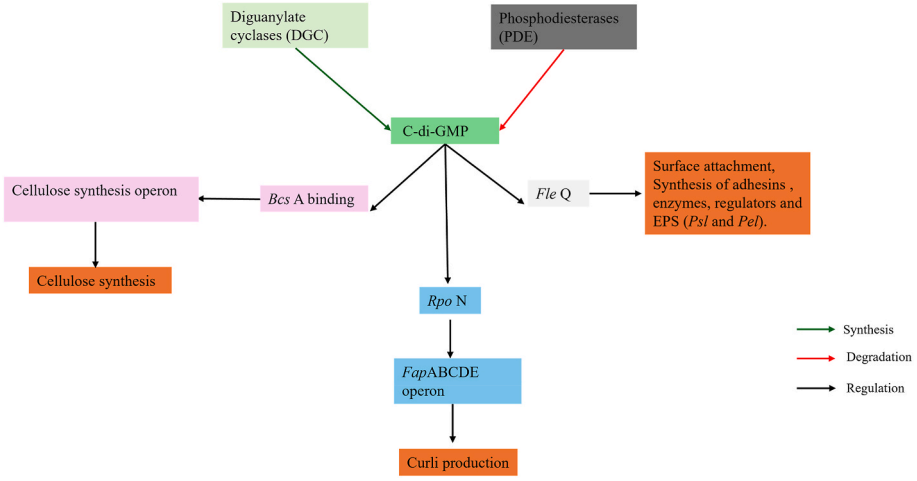

Fig. 4. The ci-di-GMP regulation pathways in biofilm EPS synthesis in pseudomonads.

operon. Fap amyloids are the main components of *P. fluorescens* PF07 biofilms and are regulated by *rpo*N and *brf*A (Guo et al., 2022). The importance of Fap amyloids in the biofilm formation is mostly discussed with pseudomonads. However, cellulose production is an important aspect of air-liquid interface biofilm-forming pseudomonads. The genes *algD, psl,* and *pel* are involved in the exopolysaccharide production of pseudomonads (Rajabi et al., 2022). FleQ is a bacterial enhancer binding protein and responds to c-di-GMP signalling by controlling surface attachment and biofilm formation, stimulating the synthesis of enzymes, regulators, adhesins, EPS, and other envelope components in a wide range of pseudomonads. FleQ also controls the flagellin synthesis and flagella production. The gene *fleN* is the anti-activator FleQ and the deletion of *fleN* leads to a hyperflagellated phenotype (Martínez-Rodríguez et al., 2023). Bacterial cellulose is also a part of biofilm formation in some gram-negative proteobacteria. *bcs* AB tandem is responsible for cellulose synthesis in *Pseudomonas* spp. biofilms. Cellulose has exceptional water retention capacity, porosity, mechanical resistance, interaction with other saccharide or proteinaceous compounds, and an architectural element for biofilms (Abidi et al., 2022). *P. putida*, *P. syringae,* and *P. fluorescens* supsp. *cellulosa* are the common cellulose producing pseudomonads (Nielsen et al., 2011).

9. Biofilm removal strategies

The conventional clean-in-place (CIP) process involves water rinse, alkali wash (saponify and remove organic material) with or without acid rinse (remove mineral deposits) and sanitizer treatments (to inactivate any remaining microorganisms) (Joseph et al., 2001). Chlorination is used in food processing environments and readily reacts with the microorganisms. However, chlorine-based cleaners lose their effectiveness when in contact with various organic substances such as milk proteins and fats and undesired accumulations of chlorine result in health risks (Pant et al., 2023). The energy and water consumption during the CIP process raises concerns. The CIP process also increases the salt load and nutrient load in the effluent, which is not environmentally friendly (Pant et al., 2023). The presence of EPS is responsible for the resistance to cleaning chemicals. Some research even suggests that biofilm is made up of 90 % EPS with polysaccharides as a major component and less than 10 % cells (Fleming et al., 2017). Biofilm disinfection depends on various factors such as the hardness of water, presence of inhibitors, concentration of cleaning chemicals and time of contact (Simões et al., 2010). Pseudomonads overproduce EPS matrix, especially polysaccharides, which were discussed in section 6. The strategies discussed here target both the cells and EPS.

9.1. Enzymes

The biofilm EPS matrix comprises proteins, polysaccharides, lipids, and eDNA. The enzymatic removal of these biofilms must target the components of the EPS matrix. The enzymes hydrolyse the biofilm EPS and convert the cells into their planktonic form which increases their susceptibility toward antimicrobials (Wang, Fletcher, et al., 2023). The biofilms of *P. aeruginosa* are hard to remove by DNase I, and the enzyme gets inactivated by the over-produced EPS matrix and proteolytic exoenzymes in mature biofilms. However, the combination of DNase I, α-amylase, and dispersin B reduced the cell counts and EPS biomass (Algburi et al., 2017). The α-amylase derived from *Bacillus subtilis* is effective against *S. aureus* and *P. aeruginosa* biofilms compared to the amylases from human saliva and sweet potato (Kalpana et al., 2012). The biofilm removal of *P. aeruginosa* by 82 % is achieved by α-amylase (Singh et al., 2016). *In vitro* studies have demonstrated the effects of dispersin B against *S. aureus, S. epidermis, A. baumanni, K. pneumoniae, E. coli* and *P. fluorescens* (Wang, Zhao, et al., 2023). Purified marine alginate enzyme (AlyP1400) can degrade the *P. aeruginosa* biofilms and enhance the cidal activity of tobramycin (Blanco-Cabra et al., 2020). PslG is a glycoside hydrolase capable of hydrolyzing Psl polysaccharide in *P. aeruginosa* in its early stages but not mature biofilms (Baker et al., 2015). $PelA_h$ is another glycoside hydrolase that targets the Pel polysaccharide in the biofilm matrix of *P. aeruginosa* and encourages significant biofilm dispersal within 24 h. However, PslG, and $PelA_h$ cannot disperse the matured biofilms (Baker et al., 2016). Treating P. *aeruginosa* biofilms with Proteinase K (2,5 and 10 μL) shows that proteinase K is biphasic and promoted or inhibited biofilm formation at different concentrations. However, the authors concluded that no antibiofilm activity was observed for Proteinase K (Eladawy et al., 2020). Mechmechani et al. (2023) combined trypsin with pepsin and carvacrol, and this combination was able to disperse the biofilms of *P. aeruginosa* and *Enterococcus faecalis*. However, the enzymatic studies were done with *P. aeruginosa*, and many other spoilage pseudomonads are found in the food industry; this treatment may not apply to all the pseudomonads. The main disadvantage of these enzymes is that when administered, they facilitate the dispersion without bactericidal activity, and this may lead to new biofilm formation from the dispersed cells (Wang, Zhao, et al., 2023). However, most of the enzymatic removal studies were done with *P. aeruginosa,* and the industrial-relevant pseudomonad biofilms need to be studied. Alkaline and enzymatic detergents improve the efficacy of disinfectants tested against strong biofilms of a poultry strain *P. fluorescens* (Merino et al., 2024). Enzymes such as DNase I, cellulase, and α-amylase, when immobilized with silver nanoparticles, can disperse the biofilms and reduce the cell viability. However, the

antibacterial activity is achieved by silver rather than the enzymes (Rubio-Canalejas et al., 2022). Combination of enzymes needs to be developed with different pH to completely remove the pseudomonad biofilms rather than single enzymes.

9.2. Ultrasound

Ultrasound affects the biofilms by three different mechanisms: mechanical effects by cavitation, chemical effects including the generation of free radicals, and heat effects by generation of local hotspots with very high temperatures (Lambert et al., 2010). A study with the removal of *P. fluorescens* in a dairy environment shows that 2 % lactic acid with ultrasound treatment can remove the biofilm cells below the detection limit (Dai, Zhang, et al., 2024). Another study with ultrasound (power>80 W) combined with mild heat treatment at 50 °C can successfully remove the biofilm of *P. fluorescens, NNB,* and a large disruption of the biofilm structure occurs at 15 min of treatment (Su et al., 2022). Ultrasound treatment on *P. fluorescens* biofilms altered around 27 metabolites involved in carbohydrate, lipid, amino acid, and nucleotide metabolism. Ultrasound at 15.79 W/cm^2 disrupted the energy and genetic information of biofilms while at 26.32 W/cm^2 resulted in disruption of the metabolic activity and osmotic pressure of biofilms (Wang et al., 2024).

9.3. Quorum-quenching molecules

Quorum-quenching (QQ) enzymes or molecules should suppress the AHL quorum-sensing molecule and cut down the cell-to-cell communication in the biofilms. A novel quorum quenching enzyme, LrsL, isolated from the red sea sediment bacteria *Labrenzia* sp. VG12 effectively suppressed the biofilm formation of *P. aeruginosa*. However, the elimination of established biofilms was not studied. The authors conclude that, with QQ enzymes, complete elimination is not possible but the amount of antimicrobials and cleaning chemicals may be lowered (Rehman et al., 2022). QQ enzymes belong to two classes: (i) AHL lactone and (ii) AHL acylases. The QQ strains isolated from a sludge membrane bioreactor, which belonged to the genera *Bacillus* and *Pseudomonas,* successfully prevented the biofilm formation of *P. aeruginosa* by 60 % (Khalid et al., 2022).

9.4. Sanitizers

Didecyldimethylammonium bromide (DDAB) and Slightly Acidic Electrolysed Water (SAEB) remove the biofilms of *P. aeruginosa*. DDAB 16 MIC and SAEW completely cleared the biofilm formation of *P. aeruginosa* on a stainless-steel surface (Li et al., 2022). Among the four sanitizers tested, benzalkonium chloride (BAC) and glycolic acid (GA) increased the less susceptible persister cells of *P. fluorescens* during regrowth. In contrast, peracetic acid (PAA) and glyoxal (GO) did not change the susceptibility of the persister cells. It is important to consider the sanitizer that should not induce a change in the antimicrobial susceptibility of persister cells. Application of ozonated water (gas inlet concentration of 20 mg/L, airflow rate of 1.0 L/min, water temperature 6 ± 1 °C) under flow conditions for *P. parancis* biofilms effectively removed 0.510 log CFU/cm^2 compared to static removal of 0.22 log CFU/cm^2 (Santos et al., 2025). Gaseous ozone application is ineffective in removing the established biofilms of *Pseudomonas* spp. (Panebianco et al., 2022).

The next generation QAC (Quaternary Ammonium Chloride) known as Decon 7 achieved approximately 4–5 log reduction in *S. aureus* and *P. aeruginosa* biofilms (Shah & Muriana, 2021). When the pseudomonad biofilms grown at 25, 10, and 4 °C were treated with sodium hypochlorite at 65 °C, the log reductions with the biofilms grown at 4 °C were lower than the biofilms grown at 25 and 10 °C (Liu et al., 2023). This shows the importance of EPS produced by pseudomonads at lower temperatures in resistance to antimicrobials. The Pel and Psl polysaccharides in *P. aeruginosa* exhibit protective functions against oxidative stress induced by sodium hypochlorite and hydrogen peroxide (H_2O_2). The interaction between the antimicrobials and Pel provides time for the biofilm cells to grow and produce detoxifying enzymes in pseudomonads (Da Cruz Nizer et al., 2024).

9.5. Biosurfactants

When the *Lactobacillus* cell-free supernatant (CFS) is supplemented with the biofilm formation medium of *P. aeruginosa,* this results in a 99.99 ± 0.003 % non-adhesion, and the application of *Lactobacillus* CFS on preformed pseudomonad biofilms results in an 83.83 ± 6.28 % decrease in metabolic activity. These results suggest the promising ability of biosurfactants of lactic acid bacteria as antiadhesive, antibiofilm activities against *P. aeruginosa* (Jeyanathan et al., 2021). Surfactant derived from *Lactiplantibacillus plantarum* reduced the biofilm formation of *P. aeruginosa* by 59.8 % and at the sub-MIC concentration QS inhibitory effects such as reduction in pyocyanin, total protease, LasA, and LasB were also observed (Patel et al., 2022). Crude biosurfactant from *L. plantarum* showed disruption in bacterial cell walls and reduction in thickness of multilayered biofilms under SEM (Patel et al., 2021). Biosurfactants from *B. niabensis* reduce growth and biofilm formation of the marine biofouling bacteria *P. stutzeri* by upregulating metabolites such as glucose, acetic acid, histidine, lactic acid, phenylalanine, uracil, and NADP+ and downregulating trehalose and histamine (Sánchez-Lozano et al., 2023).

9.6. Photodynamic inactivation (PDI)

Octyl Gallate and Blue light, when used to treat *P. fluorescens* biofilms, exhibit synergistic bactericidal and antibiofilm activity by reactive oxygen species produced by oxidative stress (Shi et al., 2022). Berberine, when combined with blue LED at 450 nm, can destroy the biofilm cells at 150 and 500 μg/mL concentrations by oxidative stress, while without blue LED, the effects on biofilm removal are significantly lower (Safai et al., 2022). The photodynamic inactivation of *P. fluorescens* in milk showed that curcumin with blue LED at 450 nm killed around 7 LogCFU/mL cells, while riboflavin had no effect. SEM shows that photodynamic inhibition causes changes in cell shape, cell damage, and membrane rupture with cytoplasmic contents around the cells. However, this study focused on the planktonic cells in milk media, and the interference of biomolecules in the milk in cell reduction is noticed (Saraiva et al., 2024). The interference from biofilm EPS is another aspect to consider when designing PDI.

9.7. Cold atmospheric plasma (CAP)

Cold plasma is a new emerging technology proven to be efficient against even bacteria with antibiotic resistance (Mai-Prochnow et al., 2015). Cold plasma is a mixture of photons, electrons, charged ions, atoms, free radicals, and excited molecules with bactericidal activities at temperatures below 40 °C (Mai-Prochnow et al., 2021). The single and dual species biofilms of *P. aeruginosa* and *B. cereus* revealed that *Bacillus* (38 % remained after 120 s of CAP treatment) is more tolerant to cold plasma than *P. aeruginosa* (33 % remained after 120 s of CAP treatment) However, in the dual species biofilms the tolerance was higher (36 % after 120s of CAP treatment) (Lavrikova, Janda, Bujdáková, & Hensel, 2025). Moisture in the biofilms reduces the efficiency of CAP treatment. Desiccation before treatment is recommended to have better penetration of reactive species such as O_2 and NO_2 generated by cold plasma (Lavrikova et al., 2025). When the biofilms were allowed to regrow after being treated with CAP for 300 s resulted in regrowth of the *P. aeruginosa* biofilms occurred both in wet and dry conditions (*B. cereus* failed to regrow under dry conditions). The author recommended additional treatments after CAP to ensure complete biofilm removal (Lavrikova et al., 2025).

10. Pseudomonad biofilm footprints

Biosurfactants, quorum-quenching (QQ) molecules, and photodynamic inactivation (PDI) mostly target the prevention of biofilm formation in pseudomonads. Other strategies, such as sanitizers, ultrasound, and enzymes, focus on preventing and removing biofilms. Among these strategies, enzymes target biofilm EPS rather than the cells (Fig. 5A). However, the enzymes cannot completely disperse the biofilms (Baker et al., 2015, 2016). The cleansers and sanitizers can remove the cells, but the remaining EPS footprints can accommodate new occupant cells and encourage new microcolonies and biofilm formation (Fig. 5B). The term "Footprints" represents the polymeric material left on the surface while the bacterial cells have been removed. The cell outlines can be seen after cell removal, and the polymeric material is clumped together due to hydrophobicity (Neu & Marshall, 1991). didecyldimethylammonium bromide (DDAB) combined with slightly acidic electrolysed water (SAEW), showed less prominent FTIR peaks for polysaccharides, proteins, and lipids than control biofilms. This confirms the remaining EPS footprints after cleaning (Li et al., 2022). The air-liquid interface biofilms of *P. fluorescens* Pf1 after CIP resulted in dead cells and EPS debris found on the coupons with no viable cells (Jha et al., 2020). A study with multispecies biofilms cleaned with antimicrobials resulted in early and aggressive regrowth of subsequent biofilms (Han et al., 2019). *P. aeruginosa* biofilms treated with 3 % hydrogen peroxide (H_2O_2) and peracetic acid (PAA)were able to reduce the biofilm cell numbers to undetectable levels. However, with SEM, the EPS residues that remained on the surface were seen. The EPS remnants significantly increased the proliferation of cells in the next 24 h. This observation suggests that EPS produced by pseudomonads is the main culprit in biofilm regrowth (Deng et al., 2025). The above-mentioned studies mentioned EPS remnants. The viscoelastic properties of EPS produced by pseudomonads remained unchanged when mechanical, chemical, and both forces were applied. The EPS produced by most of the pseudomonads can recover from the damage (Lieleg et al., 2011). The FTIR observations showed peaks for proteins, polysaccharides, and phospholipids after cleaning with didecyldimethylammonium bromide (DDAB) and slightly acidic electrolysed water (SAEW), indicating the EPS's complexity remained the same after cleaning with DDAB and SAEW (Li et al., 2022). However, there is no information about the eDNA present in the footprints. Microbubblers have the potential to invade, deform, and displace EPS by 99.9 %. Self-locomotive antimicrobial microrobot known as SLAM. SLAM is a rod-shaped diatom particle with MnO_2 (Deng et al., 2025). SLAM, when combined with peracetic acid (PAA) and hydrogen peroxide (H_2O_2), shows promising results against *P. aeruginosa* biofilms and prevents regrowth over time by reducing the EPS volume and preventing the regrowth of biofilms for more than two months (Deng et al., 2025). Pseudomonads are strong EPS producers and can accommodate other pathogens in their EPS matrix as EPS can be exploited as a public good. Thus, the removal of their EPS is important to keep the food processing surface clean. Not only pseudomonads, but many other strong EPS producers could leave the biofilm footprints.

11. Future research

Pseudomonads are public good producers and can accommodate other bacteria in their matrix. Thus, the biofilm eradication studies in the future should focus on community biofilms. The traditional sequential treatment of cleaning chemicals targets both cells and EPS. Improved strategies need to be developed to target the biofilm EPS

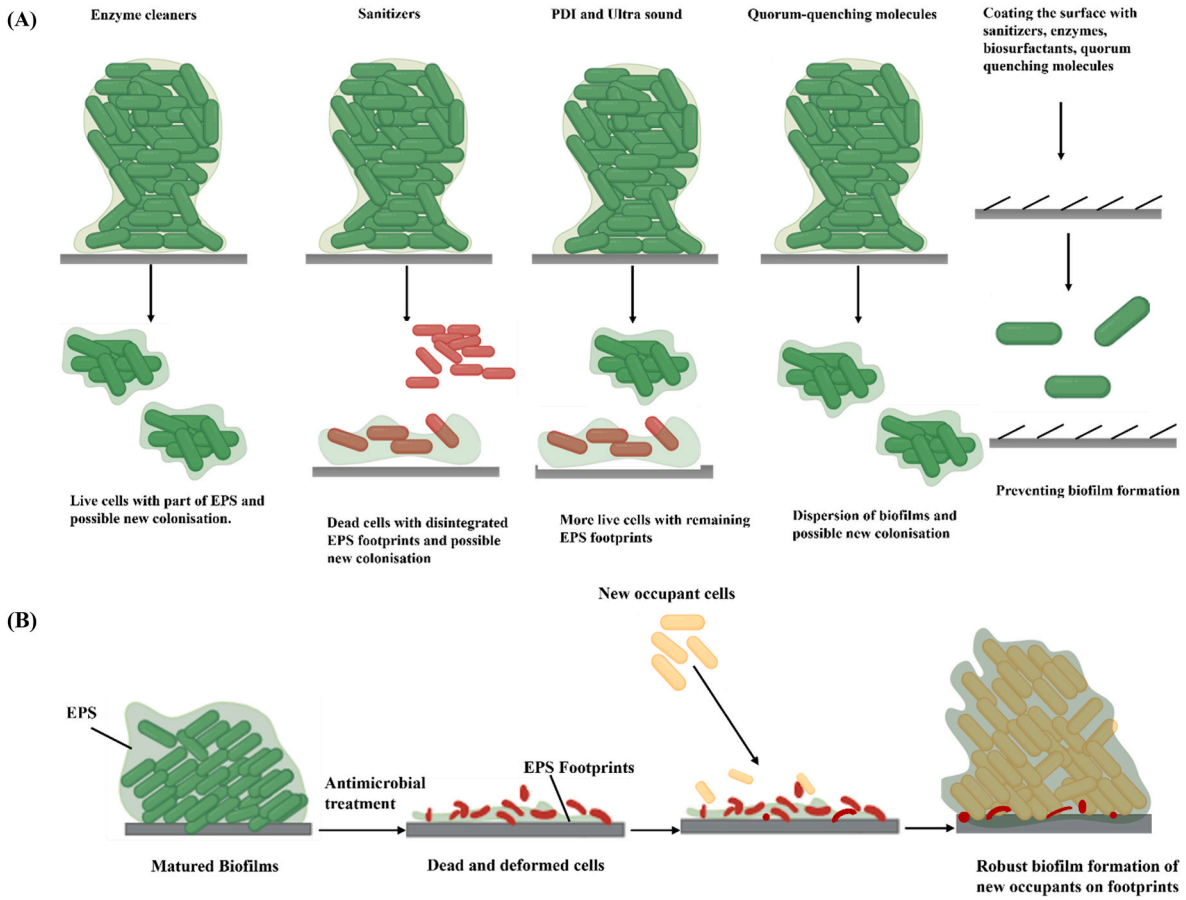

Fig. 5. (A) Biofilm prevention and eradication strategies of pseudomonads. (B) Biofilm footprints of pseudomonads after antimicrobial treatment or cleaning and the robust biofilm formation on the footprints by new occupant cells (Created in https://BioRender.com).

footprints left behind after cleaning. In a study on multispecies biofilm removal, sequential treatment steps included alkali, surfactant 1, acid, enzyme, surfactant 2, and sanitizers (Singh & Anand, 2022). Among the tested bacteria in the multispecies consortia, around 1.13 ± 0.03 logCFU/cm^2 *Bacillus* sp cells were remaining was difficult to eliminate (Singh & Anand, 2022). Processing equipment modifications with removable parts may enhance the biofilm removal. Bacteriophages show promising effects in killing biofilm cells and disrupting the EPS matrix. However, most studies are limited to laboratory conditions; scaling up and standardisation need to be developed (Yin et al., 2022). Enzyme immobilization can reduce the particle size, increase the zeta potential, and enhance the penetrability of the enzymes (Rubio-Canalejas et al., 2022). However, the immobilized enzymes still target the EPS matrix and disperse the live cells. To achieve complete elimination of biofilms, many studies suggest combined treatments. Combined treatment of enzymes with ultrasound, shear stress, chelating agents, buffers, surfactants, and detergents can achieve complete removal. However, cost-effectiveness is the biggest burden here (Nahar et al., 2018). Physical, chemical, and biological disinfection methods in combination with each other are also a strategy to combat the pseudomonad biofilms. Formulation of such strategies needs more research to find the right combinations that eradicate biofilm (Yuan et al., 2021). Nanocomposites can eliminate pseudomonad biofilms by inhibiting bacterial adhesion to the surface, disrupting the EPS matrix, and dispersing the bacterial cells. Cost-effective production and biocompatibility need to be addressed (Omran et al., 2024).

12. Conclusion

Pseudomonads are strong EPS producers, and the incomplete removal of the biofilm results in continuous contamination in food processing environments. Despite there are so many eradication methods to control the biofilm removal of psychrotrophic pseudomonads, the removal is complicated due to exopolysaccharides and matrix overproduction. Herein, the biofilm formation of psychrotrophic pseudomonads and their EPS matrix is discussed. This review compared the different eradication strategies focused on cells and EPS matrix and found that the strategies need to be combined for complete biofilm removal. This review emphasizes the need for more studies to investigate the cost-effective production, minimizing the adverse effects and safety on food matrices of strategies targeting both biofilm cells and the EPS matrix.

CRediT authorship contribution statement

Srinithi Muthuraman: Writing – review & editing, Writing – original draft, Conceptualization. **Jon Palmer:** Writing – review & editing, Supervision. **Steve Flint:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Abdel-Mawgoud, A. M., Lépine, F., & Déziel, E. (2010). Rhamnolipids: Diversity of structures, microbial origins and roles. *Applied Microbiology and Biotechnology, 86*(5), 1323–1336. https://doi.org/10.1007/s00253-010-2498-2

Abidi, W., Torres-Sánchez, L., Siroy, A., & Krasteva, P. V. (2022). Weaving of bacterial cellulose by the *bcs* secretion systems. *FEMS Microbiology Reviews, 46*(2), Article fuab051. https://doi.org/10.1093/femsre/fuab051

Ahmed, A., & Hassan, M. (2024). Monitoring the prevalence of *Pseudomonas fluorescens* as a spoilage indicator in cow raw milk, teat surfaces and milk tanks. *Open Veterinary Journal, 14*(8), 1983. https://doi.org/10.5455/OVJ.2024.v14.i8.27

Algburi, A., Comito, N., Kashtanov, D., Dicks, L. M. T., & Chikindas, M. L. (2017). Control of Biofilm formation: Antibiotics and beyond. *Applied and Environmental Microbiology, 83*(3), Article e02508. https://doi.org/10.1128/AEM.02508-16, 16.

Ardré, M., Dufour, D., & Rainey, P. B. (2019). Causes and biophysical consequences of cellulose production by *Pseudomonas fluorescens* SBW25 at the air-liquid interface. *Journal of Bacteriology, 201*(18). https://doi.org/10.1128/JB.00110-19

Baker, P., Hill, P. J., Snarr, B. D., Alnabelseya, N., Pestrak, M. J., Lee, M. J., Jennings, L. K., Tam, J., Melnyk, R. A., Parsek, M. R., Sheppard, D. C., Wozniak, D. J., & Howell, P. L. (2016). Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent *Pseudomonas aeruginosa* biofilms. *Science Advances, 2*(5), Article e1501632. https://doi.org/10.1126/sciadv.1501632

Baker, P., Whitfield, G. B., Hill, P. J., Little, D. J., Pestrak, M. J., Robinson, H., Wozniak, D. J., & Howell, P. L. (2015). Characterization of the *Pseudomonas aeruginosa* glycoside hydrolase PslG reveals that its levels are critical for Psl polysaccharide biosynthesis and biofilm formation. *Journal of Biological Chemistry, 290*(47), 28374–28387. https://doi.org/10.1074/jbc.M115.674929

Bhadra, S., Chettri, D., & Kumar Verma, A. (2023). Biosurfactants: Secondary metabolites involved in the process of bioremediation and biofilm removal. *Applied Biochemistry and Biotechnology, 195*(9), 5541–5567. https://doi.org/10.1007/s12010-022-03951-3

Blanco-Cabra, N., Paetzold, B., Ferrar, T., Mazzolini, R., Torrents, E., Serrano, L., & Lluch-Senar, M. (2020). Characterization of different alginate lyases for dissolving *Pseudomonas aeruginosa* biofilms. *Scientific Reports, 10*(1), 9390. https://doi.org/10.1038/s41598-020-66293-2

Bruzaroski, S. R., Correia, S. D. S., Devara, L. F. D. S., Poli-Frederico, R. C., Fagnani, R., & Santana, E. H. W. D. (2023). Influence of the storage temperature of raw sheep milk on the spoilage potential of *Pseudomonas* spp. *Small Ruminant Research, 224*, Article 106998. https://doi.org/10.1016/j.smallrumres.2023.106998

Caldera, L., Franzetti, L., Van Coillie, E., De Vos, P., Stragier, P., De Block, J., & Heyndrickx, M. (2016). Identification, enzymatic spoilage characterization and proteolytic activity quantification of *Pseudomonas* spp. isolated from different foods. *Food Microbiology, 54*, 142–153. https://doi.org/10.1016/j.fm.2015.10.004

Celik, G. Y., Aslim, B., & Beyatli, Y. (2008). Characterization and production of the exopolysaccharide (EPS) from *Pseudomonas aeruginosa* G1 and Pseudomonas putida G12 strains. *Carbohydrate Polymers, 73*(1), 178–182. https://doi.org/10.1016/j.carbpol.2007.11.021

Chauhan, M., Kimothi, A., Sharma, A., & Pandey, A. (2013). Cold adapted Pseudomonas: Ecology to biotechnology. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2023.1218708

Chen, J., Sun, Z., Jin, J., Wang, F., Yang, Q., Yu, H., Yu, J., & Wang, Y. (2023). Role of siderophore in *Pseudomonas fluorescens* biofilm formation and spoilage potential function. *Food Microbiology, 109*, Article 104151. https://doi.org/10.1016/j.fm.2022.104151

Chowdhury, M. A. H., Reem, C. S. A., Ashrafudoulla, M., Rahman, M. A., Shaila, S., Jie-Won Ha, A., & Ha, S. D. (2025). Role of advanced cleaning and sanitation techniques in biofilm prevention on dairy equipment. *Comprehensive Reviews in Food Science and Food Safety, 24*(3), Article e70176. https://doi.org/10.1111/1541-4337.70176

Chung, J., Eisha, S., Park, S., Morris, A. J., & Martin, I. (2023). How three self-secreted biofilm exopolysaccharides of *Pseudomonas aeruginosa, Psl, Pel*, and alginate, can each be exploited for antibiotic adjuvant effects in cystic fibrosis lung infection. *International Journal of Molecular Sciences, 24*(10), 8709. https://doi.org/10.3390/ijms24108709

Cieplik, F., Tabenski, L., Buchalla, W., & Maisch, T. (2014). Antimicrobial photodynamic therapy for inactivation of biofilms formed by oral key pathogens. *Frontiers in Microbiology, 5*. https://doi.org/10.3389/fmicb.2014.00405

Colagiorgi, A., Di Ciccio, P., Zanardi, E., Ghidini, S., & Ianieri, A. (2016). A look inside the *Listeria monocytogenes* biofilms extracellular matrix. *Microorganisms, 4*(3), 22. https://doi.org/10.3390/microorganisms4030022

Da Cruz Nizer, W. S., Allison, K. N., Adams, M. E., Vargas, M. A., Ahmed, D., Beaulieu, C., Raju, D., Cassol, E., Howell, P. L., & Overhage, J. (2024). The role of exopolysaccharides Psl and Pel in resistance of *Pseudomonas aeruginosa* to the oxidative stressors sodium hypochlorite and hydrogen peroxide. *Microbiology Spectrum, 12*(10), e00922–e00924. https://doi.org/10.1128/spectrum.00922-24

Dai, J., Fang, L., Wu, Y., Liu, B., Cheng, X., Yao, M., & Huang, L. (2022). Effects of exogenous AHLs on the spoilage characteristics of *Pseudomonas koreensis* PS1. *Journal of Food Science, 87*(2), 819–832. https://doi.org/10.1111/1750-3841.16038

Dai, J., Luo, W., Hu, F., & Li, S. (2024). *In vitro* inhibition of *Pseudomonas aeruginosa* PAO1 biofilm formation by DZ2002 through regulation of extracellular DNA and alginate production. *Frontiers in Cellular and Infection Microbiology, 13*, Article 1333773. https://doi.org/10.3389/fcimb.2023.1333773

Dai, H., Zhang, Y., Xu, Z., Stoteyome, T., & Yuan, L. (2024). Ultrasound promoted the inactivation efficacy of lactic acid against calcium-mediated biofilm formation by *Pseudomonas fluorescens*. *International Journal of Dairy Technology, 77*, 773–783. https://doi.org/10.1111/1471-0307.13108

Dawan, J., Zhang, S., & Ahn, J. (2025). Recent advances in biofilm control technologies for the food industry. *Antibiotics, 14*(3), 254. https://doi.org/10.3390/antibiotics14030254

De Melo, W. C., Avci, P., De Oliveira, M. N., Gupta, A., Vecchio, D., … Hamblin, M. R. (2013). Photodynamic inactivation of biofilm: Taking a lightly colored approach to stubborn infection. *Expert Review of Anti-Infective Therapy, 11*(7), 669–693. https://doi.org/10.1586/14787210.2013.811861

Del Olmo, A., Calzada, J., & Nuñez, M. (2018). The blue discoloration of fresh cheeses: A worldwide defect associated to specific contamination by *Pseudomonas fluorescens*. *Food Control, 86*, 359–366. https://doi.org/10.1016/j.foodcont.2017.12.001

Deng, Y.-H., Lee, J. H., Kim, M.-J., & Kong, H. (2025). Biofilm comes back: Controlling regrowth by mitigating the cell-matrix interaction. *Chemical Engineering Journal, 508*, Article 160947. https://doi.org/10.1016/j.cej.2025.160947

Di Martino, P. (2018). Bacterial adherence: Much more than a bond. *AIMS Microbiol., 4* (3), 563–566. https://doi.org/10.3934/microbiol.2018.3.563

Díaz De Rienzo, M. A., Banat, I. M., Dolman, B., Winterburn, J., & Martin, P. J. (2015). Sophorolipid biosurfactants: Possible uses as antibacterial and antibiofilm agent. *New Biotech, 32*(6), 720–726. https://doi.org/10.1016/j.nbt.2015.02.009

Dong, Q., Sun, L., Fang, T., Wang, Y., Li, Z., Wang, X., Wu, M., & Zhang, H. (2022). Biofilm Formation of *Listeria monocytogenes* and *Pseudomonas aeruginosa* in a simulated chicken processing environment. *Foods, 11*(13), 1917. https://doi.org/10.3390/foods11131917

Drescher, K., Dunkel, J., Nadell, C. D., Van Teeffelen, S., Grnja, I., Wingreen, N. S., Stone, H. A., & Bassler, B. L. (2016). Architectural transitions in *Vibrio cholerae* biofilms at single-cell resolution. *Proceedings of the National Academy of Sciences, 113* (14). https://doi.org/10.1073/pnas.1601702113

Du, B., Lu, M., Liu, H., Wu, H., Zheng, N., Zhang, Y., Zhao, S., Zhao, Y., Gao, T., & Wang, J. (2023). *Pseudomonas* isolates from raw milk with high level proteolytic activity display reduced carbon substrate utilization and higher levels of antibiotic resistance. *Lebensmittel-Wissenschaft und -Technologie, 181*, Article 114766. https://doi.org/10.1016/j.lwt.2023.114766

Dueholm, M. S., Petersen, S. V., Sønderkær, M., Larsen, P., Christiansen, G., Hein, K. L., … Otzen, D. E. (2010). Functional amyloid in *Pseudomonas*. *Molecular Microbiology, 77*(4), 1009–1020. https://doi.org/10.1111/j.1365-2958.2010.07269.x

Eladawy, M., El-Mowafy, M., El-Sokkary, M. M. A., & Barwa, R. (2020). Effects of lysozyme, proteinase K, and cephalosporins on biofilm Formation by clinical isolates of *Pseudomonas aeruginosa*. *Interdiscipl. Perspectives on Infectious Dis., 2020*, 1–9. https://doi.org/10.1155/2020/6156720

Erriu, M., Blus, C., Szmukler-Moncler, S., Buogo, S., Levi, R., Barbato, G., Madonnaripa, D., Denotti, G., Piras, V., & Orrù, G. (2014). Microbial biofilm modulation by ultrasound: Current concepts and controversies. *Ultrasonics Sonochemistry, 21*(1), 15–22. https://doi.org/10.1016/j.ultsonch.2013.05.011

Extracellular polymeric substances, a key element in understanding biofilm phenotype. AIMS Microbiol.

Fazli, M., Almblad, H., Rybtke, M. L., Givskov, M., Eberl, L., & Tolker-Nielsen, T. (2014). Regulation of biofilm formation in *Pseudomonas* and *Burkholderia* species. *Environmental Microbiology, 16*(7), 1961–1981. https://doi.org/10.1111/1462-2920.12448

Fernandes, S., Gomes, I. B., Simões, M., & Simões, L. C. (2024). Novel chemical-based approaches for biofilm cleaning and disinfection. *Current Opinion in Food Science, 55*, Article 101124. https://doi.org/10.1016/j.cofs.2024.101124

Fleming, D., Chahin, L., & Rumbaugh, K. (2017). Glycoside hydrolases degrade polymicrobial bacterial biofilms in wounds. *Antimicrobial Agents and Chemotherapy, 61*(2), Article e01998. https://doi.org/10.1128/AAC.01998-16, 16.

Flemming, H.-C., & Wingender, J. (2010). The biofilm matrix. *Nature Reviews Microbiology, 8*(9), 623–633. https://doi.org/10.1038/nrmicro2415

Gagnon, G. A., Rand, J. L., O'Leary, K. C., Rygel, A. C., Chauret, C., & Andrews, R. C. (2005). Disinfectant efficacy of chlorite and chlorine dioxide in drinking water biofilms. *Water Research, 39*(9), 1809–1817. https://doi.org/10.1016/j.watres.2005.02.004

Ghafoor, A., Jordens, Z., & Rehm, B. H. A. (2013). Role of PelF in Pel polysaccharide biosynthesis in *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology, 79* (9), 2968–2978. https://doi.org/10.1128/AEM.03666-12

Gjermansen, M., Nilsson, M., Yang, L., & Tolker-Nielsen, T. (2010). Characterization of starvation-induced dispersion in *Pseudomonas putida* biofilms: Genetic elements and molecular mechanisms. *Molecular Microbiology, 75*(4), 815–826. https://doi.org/10.1111/j.1365-2958.2009.06793.x

Guadarrama-Orozco, K. D., Perez-Gonzalez, C., Kota, K., Cocotl-Yañez, M., Jiménez-Cortés, J. G., Díaz-Guerrero, M., Hernández-Garnica, M., Munson, J., Cadet, F., López-Jácome, L. E., Estrada-Velasco, Á. Y., Fernández-Presas, A. M., & García-Contreras, R. (2023). To cheat or not to cheat: Cheatable and non-cheatable virulence factors in *Pseudomonas aeruginosa*. *FEMS Microbiology Ecology, 99*(11), Article fiad128. https://doi.org/10.1093/femsec/fiad128

Guo, M., Tan, S., Wu, Y., Zheng, C., Du, P., Zhu, J., Sun, A., & Liu, X. (2024). BrfA functions as a bacterial enhancer-binding protein to regulate functional amyloid fap-dependent biofilm formation in *Pseudomonas fluorescens* by sensing cyclic diguanosine monophosphate. *Microbiological Research, 287*, Article 127864. https://doi.org/10.1016/j.micres.2024.127864

Guo, M., Tan, S., Zhu, J., Sun, A., Du, P., & Liu, X. (2022). Genes involved in biofilm matrix formation of the food spoiler *Pseudomonas fluorescens* PF07. *Frontiers in Microbiology, 13*, Article 881043. https://doi.org/10.3389/fmicb.2022.881043

Guttenplan, S. B., & Kearns, D. B. (2013). Regulation of flagellar motility during biofilm formation. *FEMS Microbiology Reviews, 37*(6), 849–871. https://doi.org/10.1111/1574-6976.12018

Han, Q., Jiang, Y., Brandt, B. W., Yang, J., Chen, Y., Buijs, M. J., Crielaard, W., Cheng, L., & Deng, D. (2019). Regrowth of Microcosm biofilms on titanium surfaces after various antimicrobial treatments. *Frontiers in Microbiology, 10*, 2693. https://doi.org/10.3389/fmicb.2019.02693

Harimawan, A., & Ting, Y.-P. (2016). Investigation of extracellular polymeric substances (EPS) properties of *P. aeruginosa* and *B. subtilis* and their role in bacterial adhesion. *Colloids and Surfaces B: Biointerfaces, 146*, 459–467. https://doi.org/10.1016/j.colsurfb.2016.06.039

Hinsa, S. M., Espinosa-Urgel, M., Ramos, J. L., & O'Toole, G. A. (2003). Transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas fluorescens* WCS365 requires an ABC transporter and a large secreted protein. *Molecular Microbiology, 49*(4), 905–918. https://doi.org/10.1046/j.1365-2958.2003.03615

Hoda, M. (2012). Proteolytic and lipolytic activities of Pseudomonas spp. isolated from pasteurized milk. *African Journal of Food Science, 6*(6). https://doi.org/10.5897/AJFS11.152

Jennings, L. K., Storek, K. M., Ledvina, H. E., Coulon, C., Marmont, L. S., Sadovskaya, I., Secor, P. R., Tseng, B. S., Scian, M., Filloux, A., Wozniak, D. J., Howell, P. L., & Parsek, M. R. (2015). *Pel* is a cationic exopolysaccharide that cross-links extracellular DNA in the *Pseudomonas aeruginosa* biofilm matrix. *Proceedings of the National Academy of Sciences of the United States of America, 112*(36), 11353–11358. https://doi.org/10.1073/pnas.1503058112

Jeyanathan, A., Ramalhete, R., Blunn, G., Coathup, M., & Sammons, R. (2021). Lactobacillus cell-free supernatant as a novel bioagent and biosurfactant against *Pseudomonas aeruginosa* in the prevention and treatment of orthopedic implant infection. *Journal of Biomedical Materials Research Part B: Applied Biomaterials, 109* (12), 1634–1643. https://doi.org/10.1002/jbm.b.34821

Jha, P. K., Dallagi, H., Richard, E., Benezech, T., & Faille, C. (2020). Formation and resistance to cleaning of biofilms at air-liquid-wall interface. Influence of bacterial strain and material. *Food Control, 118*, Article 107384. https://doi.org/10.1016/j.foodcont.2020.107384

Jimoh, A. A., Booysen, E., Van Zyl, L., & Trindade, M. (2023). Do biosurfactants as anti-biofilm agents have a future in industrial water systems? *Frontiers in Bioengineering and Biotechnology, 11*. https://doi.org/10.3389/fbioe.2023.1244595

Jones, I. A., & Joshi, L. T. (2021). Biocide use in the antimicrobial era: A review. *Molecules, 26*(8), 2276. https://doi.org/10.3390/molecules26082276

Joseph, B., Otta, S. K., Karunasagar, I., & Karunasagar, I. (2001). Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. *International Journal of Food Microbiology, 64*(3), 367–372. https://doi.org/10.1016/S0168-1605(00)00466-9

Julou, T., Mora, T., Guillon, L., Croquette, V., Schalk, I. J., Bensimon, D., & Desprat, N. (2013). Cell–cell contacts confine public goods diffusion inside *Pseudomonas aeruginosa* clonal microcolonies. *Proceedings of the National Academy of Sciences, 110* (31), 12577–12582. https://doi.org/10.1073/pnas.1301428110

Kalpana, B. J., Aarthy, S., & Pandian, S. K. (2012). Antibiofilm activity of α-Amylase from *Bacillus subtilis* S8-18 against biofilm forming human bacterial pathogens. *Applied Biochemistry and Biotechnology, 167*(6), 1778–1794. https://doi.org/10.1007/s12010-011-9526-2

Karygianni, L., Ren, Z., Koo, H., & Thurnheer, T. (2020). Biofilm matrixome: Extracellular components in structured microbial communities. *Trends in Microbiology, 28*(8), 668–681. https://doi.org/10.1016/j.tim.2020.03.016

Khalid, S. J., Ain, Q., Khan, S. J., Jalil, A., Siddiqui, M. F., Ahmad, T., Badshah, M., & Adnan, F. (2022). Targeting Acyl Homoserine Lactones (AHLs) by the quorum quenching bacterial strains to control biofilm formation in *Pseudomonas aeruginosa*. *Saudi Journal of Biological Sciences, 29*(3), 1673–1682. https://doi.org/10.1016/j.sjbs.2021.10.064

Kolbeck, S., Abele, M., Hilgarth, M., & Vogel, R. F. (2021). Comparative proteomics reveals the anaerobic lifestyle of meat-spoiling *pseudomonas* species. *Frontiers in Microbiology, 12*, Article 664061. https://doi.org/10.3389/fmicb.2021.664061

Krzyżek, P. (2019). Challenges and limitations of anti-quorum sensing therapies. *Frontiers in Microbiology, 10*. https://doi.org/10.3389/fmicb.2019.02473

Kumar, H., Franzetti, L., Kaushal, A., & Kumar, D. (2019). *Pseudomonas fluorescens*: A potential food spoiler and challenges and advances in its detection. *Annals of Microbiology, 69*(9), 873–883. https://doi.org/10.1007/s13213-019-01501-7

Kunzler, M., Schlechter, R. O., Schreiber, L., & Remus-Emsermann, M. N. P. (2024). Hitching a ride in the phyllosphere: Surfactant production of *Pseudomonas* spp. causes Co-swarming of *Pantoea eucalypti* 299R. *Microbial Ecology, 87*(1), 62. https://doi.org/10.1007/s00248-024-02381-4

Lalucat, J., Gomila, M., Mulet, M., Zaruma, A., & García-Valdés, E. (2022). Past, present and future of the boundaries of the *Pseudomonas* genus: Proposal of Stutzerimonas gen. Nov. *Systematic & Applied Microbiology, 45*(1), Article 126289. https://doi.org/10.1016/j.syapm.2021.126289

Lambert, N., Rediers, H., Hulsmans, A., Joris, K., Declerck, P., De Laedt, Y., & Liers, S. (2010). Evaluation of ultrasound technology for the disinfection of process water and the prevention of biofilm formation in a pilot plant. *Water Science and Technology, 61*(5), 1089–1096. https://doi.org/10.2166/wst.2010.735

Lau, G. W., Hassett, D. J., Ran, H., & Kong, F. (2004). The role of pyocyanin in *Pseudomonas aeruginosa* infection. *Trends in Molecular Medicine, 10*(12), 599–606. https://doi.org/10.1016/j.molmed.2004.10.002

Laue, H., Schenk, A., Li, H., Lambertsen, L., Neu, T. R., Molin, S., & Ullrich, M. S. (2006). Contribution of alginate and levan production to biofilm formation by *Pseudomonas syringae*. *Microbiology, 152*(10), 2909–2918. https://doi.org/10.1099/mic.0.28875-0

Lavrikova, A., Janda, M., Bujdáková, H., & Hensel, K. (2025). Eradication of single- and mixed-species biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus* by pulsed streamer Corona discharge cold atmospheric plasma. *Science of the Total Environment, 916*, Article 178184. https://doi.org/10.1016/j.scitotenv.2024.178184

Le Mauff, F., Razvi, E., Reichhardt, C., et al. (2022). The *Pel* polysaccharide is predominantly composed of a dimeric repeat of α-1,4 linked galactosamine and *N*-acetylgalactosamine. *Communications Biology, 5*, 502. https://doi.org/10.1038/s42003-022-03453-2

Li, Y., Wang, H., Zheng, X., Li, Z., Wang, M., Luo, K., Zhang, C., Xia, X., Wang, Y., & Shi, C. (2022). Didecyldimethylammonium bromide: Application to control biofilms of Staphylococcus aureus and *Pseudomonas aeruginosa* alone and in combination with

slightly acidic electrolyzed water. *Food Research International, 157*, Article 111236. https://doi.org/10.1016/j.foodres.2022.111236
Lieleg, O., Caldara, M., Baumgärtel, R., & Ribbeck, K. (2011). Mechanical robustness of *Pseudomonas aeruginosa* biofilms. *Soft Matter, 7*(7), 3307. https://doi.org/10.1039/c0sm01467b
Liu, Y., Xie, J., Zhao, L., Qian, Y., Zhao, Y., & Liu, X. (2015). Biofilm formation characteristics of *Pseudomonas lundensis* isolated from meat. *Journal of Food Science, 80*(12). https://doi.org/10.1111/1750-3841.13142
Liu, Z., Hossain, S. S., Morales Moreira, Z., & Haney, C. H. (2022). Putrescine and its metabolic precursor arginine promote biofilm and c-di-GMP synthesis in *Pseudomonas aeruginosa*. *Journal of Bacteriology, 204*(1), Article e00297. https://doi.org/10.1128/JB.00297-21, 21.
Liu, J., Wu, S., Feng, L., Wu, Y., & Zhu, J. (2023). Extracellular matrix affects mature biofilm and stress resistance of psychrotrophic spoilage *Pseudomonas* at cold temperature. *Food Microbiology, 112*, Article 104214. https://doi.org/10.1016/j.fm.2023.104214
Liu, X., Ye, Y., Zhu, Y., Wang, L., Yuan, L., Zhu, J., & Sun, A. (2021). Involvement of RpoN in regulating motility, biofilm, resistance, and spoilage potential of *Pseudomonas fluorescens*. *Frontiers in Microbiology, 12*, Article 641844. https://doi.org/10.3389/fmicb.2021.641844
Loarca, D., Díaz, D., Quezada, H., Guzmán-Ortiz, A. L., Rebollar-Ruiz, A., Presas, A. M. F., Ramírez-Peris, J., Franco-Cendejas, R., Maeda, T., Wood, T. K., & García-Contreras, R. (2019). Seeding public goods is essential for maintaining cooperation in *Pseudomonas aeruginosa*. *Frontiers in Microbiology, 10*, 2322. https://doi.org/10.3389/fmicb.2019.02322
Machado, S. G., Baglinière, F., Marchand, S., Van Coillie, E., Vanetti, M. C. D., De Block, J., & Heyndrickx, M. (2017). The biodiversity of the microbiota producing heat-resistant enzymes responsible for spoilage in processed bovine milk and dairy products. *Frontiers in Microbiology, 8*. https://doi.org/10.3389/fmicb.2017.00302
Mai-Prochnow, A., Bradbury, M., Ostrikov, K., & Murphy, A. B. (2015). *Pseudomonas aeruginosa* biofilm response and resistance to cold atmospheric pressure plasma is linked to the redox-active molecule phenazine. *PLoS One, 10*(6), Article e0130373. https://doi.org/10.1371/journal.pone.0130373
Mai-Prochnow, A., Zhou, R., Zhang, T., Ostrikov, K., Mugunthan, S., Rice, S. A., & Cullen, P. J. (2021). Interactions of plasma-activated water with biofilms: Inactivation, dispersal effects and mechanisms of action. *Npj Biofilms and Microbiomes, 7*(1), 11. https://doi.org/10.1038/s41522-020-00180-6
Mann, E. E., & Wozniak, D. J. (2012). *Pseudomonas* biofilm matrix composition and niche biology. *FEMS Microbiology Reviews, 36*(4), 893–916. https://doi.org/10.3389/fcimb.2023.1333773
Martínez-Gil, M., Quesada, J. M., Ramos-González, M. I., Soriano, M. I., De Cristóbal, R. E., & Espinosa-Urgel, M. (2013). Interplay between extracellular matrix components of *Pseudomonas putida* biofilms. *Research in Microbiology, 164*(5), 382–389. https://doi.org/10.1016/j.resmic.2013.03.021
Martínez-Rodríguez, L., López-Sánchez, A., García-Alcaide, A., Govantes, F., & Gallegos, M.-T. (2023). FleQ, FleN and c-di-GMP coordinately regulate cellulose production in *Pseudomonas syringae* pv. Tomato DC3000. *Frontiers in Molecular Biosciences, 10*, Article 1155579. https://doi.org/10.3389/fmolb.2023.1155579
McDougald, D., Klebensberger, J., Tolker-Nielsen, T., Webb, J. S., Conibear, T., Rice, S. A., Kirov, S. M., Matz, C., & Kjelleberg, S. (2008). Pseudomonas aeruginosa: A model for biofilm formation. https://doi.org/10.1002/9783527622009.ch9.
McDougald, D., Rice, S. A., Barraud, N., Steinberg, P. D., & Kjelleberg, S. (2012). Should we stay or should we go: Mechanisms and ecological consequences for biofilm dispersal. *Nature Reviews Microbiology, 10*(1), 39–50. https://doi.org/10.1038/nrmicro2695
Mechmechani, S., Gharsallaoui, A., Karam, L., El Omari, K., Fadel, A., Hamze, M., & Chihib, N.-E. (2023). Pepsin and trypsin treatment combined with carvacrol: An efficient strategy to fight *Pseudomonas aeruginosa* and *Enterococcus faecalis* biofilms. *Microorganisms, 11*(1), 143. https://doi.org/10.3390/microorganisms11010143
Meng, L., Zhang, Y., Liu, H., Zhao, S., Wang, J., & Zheng, N. (2017). Characterization of *Pseudomonas* spp. and associated proteolytic properties in raw milk stored at low temperatures. *Frontiers in Microbiology, 8*, 2158. https://doi.org/10.3389/fmicb.2017.02158
Merino, N., García-Castillo, C., Berdejo, D., Pagán, E., García-Gonzalo, D., & Pagán, R. (2024). Comparative analysis of commercial cleaning and disinfection formulations and protocols for effective eradication of biofilms formed by a *Pseudomonas fluorescens* strain isolated from a poultry meat plant. *Food Control, 164*, Article 110614. https://doi.org/10.1016/j.foodcont.2024.110614
Molina, G., Pimentel, M. R., & Pastore, G. M. (2013). *Pseudomonas*: A promising biocatalyst for the bioconversion of terpenes. *Applied Microbiology and Biotechnology, 97*(5), 1851–1864. https://doi.org/10.1007/s00253-013-4701-8
Mozaheb, N., Rasouli, P., Kaur, M., Van Der Smissen, P., Larrouy-Maumus, G., & Mingeot-Leclercq, M.-P. (2023). A mildly acidic environment alters *Pseudomonas aeruginosa* virulence and causes remodeling of the bacterial surface. *Microbiology Spectrum, 11*(4), Article e04832. https://doi.org/10.1128/spectrum.04832-22, 22.
Mridha, S., & Kümmerli, R. (2022). Enforced specialization fosters mutual cheating and not division of labour in the bacterium *Pseudomonas aeruginosa*. *Journal of Evolutionary Biology, 35*(5), 719–730. https://doi.org/10.1111/jeb.14001
Muthuraman, S., Flint, S., & Palmer, J. (2025). Characterization of the extracellular polymeric substances matrix of *Pseudomonas* biofilms formed at the air-liquid interface. *Food Bioscience, 64*, Article 105918. https://doi.org/10.1016/j.fbio.2025.105918
Nahar, S., Mizan, M. F. R., Ha, A. J., & Ha, S. D. (2018). Advances and future prospects of enzyme-based biofilm prevention approaches in the food industry. *Comprehensive Reviews in Food Science and Food Safety, 17*(6), 1484–1502. https://doi.org/10.1111/1541-4337.12382
Naicker, K.-I., Kaweesa, P., Daramola, M. O., & Iwarere, S. A. (2023). Non-thermal plasma review: Assessment and improvement of feasibility as a retrofitted technology in tertiary wastewater purification. *Applied Sciences, 13*(10), 6243. https://doi.org/10.3390/app13106243
Neu, T. R., & Marshall, K. C. (1991). Microbial "footprints"—A new approach to adhesive polymers. *Biofouling, 3*(2), 101–112. https://doi.org/10.1080/08927019109378166
Nielsen, L., Li, X., & Halverson, L. J. (2011). Cell–cell and cell–surface interactions mediated by cellulose and a novel exopolysaccharide contribute to *Pseudomonas putida* biofilm formation and fitness under water-limiting conditions. *Environmental Microbiology, 13*(5), 1342–1356. https://doi.org/10.1111/j.1462-2920.2011.02432.x
O'Brien, S., Luján, A. M., Paterson, S., Cant, M. A., & Buckling, A. (2017). Adaptation to public goods cheats in *Pseudomonas aeruginosa*. *Proceedings of the Royal Society B: Biological Sciences, 284*(1859), Article 20171089. https://doi.org/10.1098/rspb.2017.1089
Omran, B. A., Tseng, B. S., & Baek, K.-H. (2024). Nanocomposites against *Pseudomonas aeruginosa* biofilms: Recent advances, challenges, and future prospects. *Microbiological Research, 282*, Article 127656. https://doi.org/10.1016/j.micres.2024.127656
O'Toole, G., Kaplan, H. B., & Kolter, R. (2000). Biofilm formation as microbial development. *Annual Review of Microbiology, 54*(1), 49–79. https://doi.org/10.1146/annurev.micro.54.1.49
Paluch, E., Rewak-Soroczyńska, J., Jędrusik, I., Mazurkiewicz, E., & Jermakow, K. (2020). Prevention of biofilm formation by quorum quenching. *Applied Microbiology and Biotechnology, 104*(5), 1871–1881. https://doi.org/10.1007/s00253-020-10349-w
Panebianco, F., Rubiola, S., Chiesa, F., Civera, T., & Di Ciccio, P. A. (2022). Effect of gaseous ozone treatment on biofilm of dairy-isolated *Pseudomonas* spp. strains. *Italian Journal of Food Safety, 11*(2). https://doi.org/10.4081/ijfs.2022.10350
Pant, K. J., Cotter, P. D., Wilkinson, M. G., & Sheehan, J. J. (2023). Towards sustainable cleaning-in-place (CIP) in dairy processing: Exploring enzyme-based approaches to cleaning in the Cheese industry. *Comprehensive Reviews in Food Science and Food Safety, 22*, 3602–3619. https://doi.org/10.1111/1541-4337.13206
Parkar, S. G., Flint, S. H., & Brooks, J. D. (2004). Evaluation of the effect of cleaning regimes on biofilms of thermophilic bacilli on stainless steel. *Journal of Applied Microbiology, 96*(1), 110–116. https://doi.org/10.1046/j.1365-2672.2003.02136.x
Patel, H., & Gajjar, D. (2022). Cell adhesion and twitching motility influence strong biofilm formation in *Pseudomonas aeruginosa*. *Biofouling, 38*(3), 235–249. https://doi.org/10.1080/08927014.2022.2054703
Patel, M., Siddiqui, A. J., Ashraf, S. A., Surti, M., Awadelkareem, A. M., Snoussi, M., Hamadou, W. S., Bardakci, F., Jamal, A., Jahan, S., Sachidanandan, M., & Adnan, M. (2022). *Lactiplantibacillus plantarum*-derived biosurfactant attenuates quorum sensing-mediated virulence and biofilm formation in *Pseudomonas aeruginosa* and *Chromobacterium violaceum*. *Microorganisms, 10*(5), 1026. https://doi.org/10.3390/microorganisms10051026
Patel, M., Siddiqui, A. J., Hamadou, W. S., Surti, M., Awadelkareem, A. M., Ashraf, S. A., Alreshidi, M., Snoussi, M., Rizvi, S. M. D., Bardakci, F., Jamal, A., Sachidanandan, M., & Adnan, M. (2021). Inhibition of bacterial adhesion and antibiofilm activities of a glycolipid biosurfactant from *Lactobacillus rhamnosus* with its physicochemical and functional properties. *Antibiotics, 10*(12), 1546. https://doi.org/10.3390/antibiotics10121546
Puga, C. H., Dahdouh, E., SanJosé, C., & Orgaz, B. (2018). *Listeria monocytogenes* colonizes *Pseudomonas fluorescens* biofilms and induces matrix over-production. *Frontiers in Microbiology, 9*, 1706. https://doi.org/10.3389/fmicb.2018.01706
Puhm, M., Ainelo, H., Kivisaar, M., & Teras, R. (2022). Tryptone in growth media enhances *Pseudomonas putida* Biofilm. *Microorganisms, 10*(3), 618. https://doi.org/10.3390/microorganisms10030618
Rajabi, H., Salimizand, H., Khodabandehloo, M., Fayyazi, A., & Ramazanzadeh, R. (2022). Prevalence of *algD* , *pslD* , *pelF* , *Ppgl* , and *PAPI-1* Genes involved in biofilm formation in clinical *Pseudomonas aeruginosa* strains. *BioMed Research International, 2022*(1), Article 1716087. https://doi.org/10.1155/2022/1716087
Rehman, Z. U., Momin, A. A., Aldehaiman, A., Irum, T., Grünberg, R., & Arold, S. T. (2022). The exceptionally efficient quorum quenching enzyme LrsL suppresses *Pseudomonas aeruginosa* biofilm production. *Frontiers in Microbiology, 13*, Article 977673. https://doi.org/10.3389/fmicb.2022.977673
Reichhardt, C. (2023). The *Pseudomonas aeruginosa* biofilm matrix protein CdrA has similarities to other fibrillar adhesin proteins. *Journal of Bacteriology, 205*(5), Article e00019. https://doi.org/10.1128/jb.00019-23, 23.
Reichhardt, C., Jacobs, H. M., Matwichuk, M., Wong, C., Wozniak, D. J., & Parsek, M. R. (2020). The versatile *Pseudomonas aeruginosa* biofilm matrix protein CdrA promotes aggregation through different extracellular exopolysaccharide interactions. *Journal of Bacteriology, 202*(19). https://doi.org/10.1128/JB.00216-20
Reichhardt, C., Wong, C., Passos Da Silva, D., Wozniak, D. J., & Parsek, M. R. (2018). CdrA interactions within the *Pseudomonas aeruginosa* biofilm matrix safeguard it from proteolysis and promote cellular packing. *mBio, 9*(5), Article e01376. https://doi.org/10.1128/mBio.01376-18, 18.
Robertson, M., Hapca, S. M., Moshynets, O., & Spiers, A. J. (2013). Air–liquid interface biofilm formation by psychrotrophic pseudomonads recovered from spoilt meat. *Antonie Van Leeuwenhoek, 103*(1), 251–259. https://doi.org/10.1007/s10482-012-9796-x
Rubio-Canalejas, A., Baelo, A., Herbera, S., Blanco-Cabra, N., Vukomanovic, M., & Torrents, E. (2022). 3D spatial organization and improved antibiotic treatment of a *Pseudomonas aeruginosa–Staphylococcus aureus* wound biofilm by nanoparticle enzyme delivery. *Frontiers in Microbiology, 13*, Article 959156. https://doi.org/10.3389/fmicb.2022.959156

Saá Ibusquiza, P., Herrera, J. J. R., Vázquez-Sánchez, D., & Cabo, M. L. (2012). Adherence kinetics, resistance to benzalkonium chloride and microscopic analysis of mixed biofilms formed by Listeria monocytogenes and *Pseudomonas putida*. *Food Control, 25*(1), 202–210. https://doi.org/10.1016/j.foodcont.2011.10.002

Safai, S. M., Khorsandi, K., & Falsafi, S. (2022). Effect of berberine and blue LED irradiation on combating biofilm of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Current Microbiology, 79*(12), 366. https://doi.org/10.1007/s00284-022-03063-5

Sánchez-Lozano, I., Muñoz-Cruz, L. C., Hellio, C., Band-Schmidt, C. J., Cruz-Narváez, Y., Becerra-Martínez, E., & Hernández-Guerrero, C. J. (2023). Metabolomic insights of biosurfactant activity from *Bacillus niabensis* against planktonic cells and biofilm of *Pseudomonas stutzeri* involved in marine biofouling. *International Journal of Molecular Sciences, 24*(4), 4249. https://doi.org/10.3390/ijms24044249

Santos, T. M., Lopes, M. E. T., De Alencar, E. R., Silva, M. V. D. A., & Machado, S. G. (2025). Ozonized water as a promising strategy to remove biofilm formed by *Pseudomonas* spp. on polyethylene and polystyrene surfaces. *Biofouling, 41*(2), 144–156. https://doi.org/10.1080/08927014.2024.2444387

Saraiva, B. B., Campanholi, K. D. S. S., Machado, R. R. B., Nakamura, C. V., Silva, A. A., Caetano, W., & Pozza, M. S. D. S. (2024). Reducing *Pseudomonas fluorescens* in milk through photodynamic inactivation using riboflavin and curcumin with 450 nm blue light-emitting diode. *International Dairy Journal, 148*, Article 105787. https://doi.org/10.1016/j.idairyj.2023.105787

Shah, K., & Muriana, P. (2021). Efficacy of a next generation Quaternary ammonium chloride sanitizer on *staphylococcus* and *Pseudomonas* biofilms and practical application in a food processing environment. *Applied Microbiology, 1*(1), 89–103. https://doi.org/10.3390/applmicrobiol1010008

Shi, Y., Jiang, L., Lin, S., Jin, W., Gu, Q., Chen, Y., Zhang, K., & Ettelaie, R. (2022). Ultra-efficient antimicrobial photodynamic inactivation system based on blue light and octyl gallate for ablation of planktonic bacteria and biofilms of *Pseudomonas fluorescens*. *Food Chemistry, 374*, Article 131585. https://doi.org/10.1016/j.foodchem.2021.131585

Silby, M. W., Winstanley, C., Godfrey, S. A. C., Levy, S. B., & Jackson, R. W. (2011). *Pseudomonas* genomes: Diverse and adaptable. *FEMS Microbiology Reviews, 35*(4), 652–680. https://doi.org/10.1111/j.1574-6976.2011.00269.x

Simões, M., Simões, L. C., & Vieira, M. J. (2010). A review of current and emergent biofilm control strategies. *LWT - Food Science and Technology, 43*(4), 573–583. https://doi.org/10.1016/j.lwt.2009.12.008

Singh, D., & Anand, S. (2022). Efficacy of a typical clean-in-place protocol against in vitro membrane biofilms. *Journal of Dairy Science, 105*(12), 9417–9425. https://doi.org/10.3168/jds.2022-21712

Singh, V., Haque, S., Singh, H., Verma, J., Vibha, K., Singh, R., Jawed, A., & Tripathi, C. K. M. (2016). Isolation, screening, and identification of novel isolates of actinomycetes from India for antimicrobial applications. *Frontiers in Microbiology, 7*. https://doi.org/10.3389/fmicb.2016.01921

Starkey, M., Hickman, J. H., Ma, L., Zhang, N., De Long, S., Hinz, A., Palacios, S., Manoil, C., Kirisits, M. J., Starner, T. D., Wozniak, D. J., Harwood, C. S., & Parsek, M. R. (2009). *Pseudomonas aeruginosa* rugose small-colony variants have adaptations that likely promote persistence in the cystic fibrosis lung. *Journal of Bacteriology, 191*(11), 3492–3503. https://doi.org/10.1128/JB.00119-09

Sterniša, M., Gradišar Centa, U., Drnovšek, A., Remškar, M., & Smole Možina, S. (2023). *Pseudomonas fragi* biofilm on stainless steel (at low temperatures) affects the survival of *Campylobacter jejuni* and *Listeria monocytogenes* and their control by a polymer molybdenum oxide nanocomposite coating. *International Journal of Food Microbiology, 394*, Article 110159. https://doi.org/10.1016/j.ijfoodmicro.2023.110159

Su, Y., Jiang, L., Chen, D., Yu, H., Yang, F., Guo, Y., Xie, Y., & Yao, W. (2022). In vitro and in silico approaches to investigate antimicrobial and biofilm removal efficacies of combined ultrasonic and mild thermal treatment against *Pseudomonas fluorescens*. *Ultrasonics Sonochemistry, 83*, Article 105930. https://doi.org/10.1016/j.ultsonch.2022.105930

Sung, K., Park, M., Chon, J., Kweon, O., & Khan, S. (2024). Unraveling the molecular dynamics of *Pseudomonas aeruginosa* biofilms at the air–liquid interface. *Future Microbiology, 19*(8), 681–696. https://doi.org/10.2217/fmb-2023-0234

Tan, X., Huang, Y., Rana, A., Singh, N., Abbey, T. C., Chen, H., Toth, P. T., & Bulman, Z. P. (2024). Optimization of an in vitro *Pseudomonas aeruginosa* biofilm model to examine antibiotic pharmacodynamics at the air-liquid interface. *Npj Biofilms and Microbiomes, 10*(1), 16. https://doi.org/10.1038/s41522-024-00483-y

Teh, K. H., Flint, S., Palmer, J., Andrewes, P., Bremer, P., & Lindsay, D. (2012). Proteolysis produced within biofilms of bacterial isolates from raw milk tankers. *International Journal of Food Microbiology, 157*(1), 28–34. https://doi.org/10.1016/j.ijfoodmicro.2012.04.008

Tribelli, P. M., & López, N. I. (2022). Insights into the temperature responses of *Pseudomonas* species in beneficial and pathogenic host interactions. *Applied Microbiology and Biotechnology, 106*(23), 7699–7709. https://doi.org/10.1007/s00253-022-12243-z

Ueda, A., & Saneoka, H. (2015). Characterization of the ability to form biofilms by plant-associated Pseudomonas Species. *Current Microbiology, 70*(4), 506–513. https://doi.org/10.1007/s00284-014-0749-7

Wagner, E. M., Fischel, K., Rammer, N., Beer, C., Palmetzhofer, A. L., Conrady, B., Roch, F.-F., Hanson, B. T., Wagner, M., & Rychli, K. (2021). Bacteria of eleven different species isolated from biofilms in a meat processing environment have diverse biofilm forming abilities. *International Journal of Food Microbiology, 349*, Article 109232. https://doi.org/10.1016/j.ijfoodmicro.2021.109232

Wang, D., Fletcher, G. C., Gagic, D., On, S. L. W., Palmer, J. S., & Flint, S. H. (2023). Comparative genome identification of accessory genes associated with strong biofilm formation in *Vibrio parahaemolyticus*. *Food Research International, 166*, Article 112605. https://doi.org/10.1016/j.foodres.2023.112605

Wang, M., Jiang, L., Liu, M., Yuan, S., Guo, Y., Yao, W., & Yu, H. (2024). Effects of ultrasound on disrupting metabolite profiles of *Pseudomonas fluorescens* biofilms cultured on the surface of lettuce. *Food Control, 155*, Article 110103. https://doi.org/10.1016/j.foodcont.2023.110103

Wang, D., Kyere, E., & Ahmed Sadiq, F. (2021). New trends in photodynamic inactivation (PDI) combating biofilms in the food industry—A review. *Foods, 10*(11), 2587. https://doi.org/10.3390/foods10112587

Wang, G., Qing, L., Tang, W., Ma, F., Wang, H., Xu, X., & Qiu, W. (2021). AprD is important for extracellular proteolytic activity, physicochemical properties and spoilage potential in meat-borne *Pseudomonas fragi*. *Food Control, 124*, Article 107868. https://doi.org/10.1016/j.foodcont.2021.107868

Wang, S., Liu, X., Liu, H., Zhang, L., Guo, Y., Yu, S., … Ma, L. Z. (2015). The exopolysaccharide Psl–eDNA interaction enables the formation of a biofilm skeleton in *Pseudomonas aeruginosa*. *Environmental Microbiology Reports, 7*(2), 330–340. https://doi.org/10.1111/1758-2229.12252

Wang, S., Zhao, Y., Breslawec, A. P., Liang, T., Deng, Z., Kuperman, L. L., & Yu, Q. (2023). Strategy to combat biofilms: A focus on biofilm dispersal enzymes. *Npj Biofilms and Microbiomes, 9*(1), 63. https://doi.org/10.1038/s41522-023-00427-y

Wehedy, S., Elshafee, A., Helal, Z., Eldin, Y., Yassin, A., & Zaki, H. (2025). Contamination of meat and its products by *Pseudomonas* species and assessment of the antibacterial effect of clove (*Syzygium aromaticum*) essential oil on multidrug-resistant *P. aeruginosa*. *Open Veterinary Journal, 0*(34), 1. https://doi.org/10.5455/OVJ.2025.v15.i4

Wickramasinghe, N. N., Hlaing, M. M., Ravensdale, J. T., Coorey, R., Chandry, P. S., & Dykes, G. A. (2020). Characterization of the biofilm matrix composition of psychrotrophic, meat spoilage pseudomonads. *Scientific Reports, 10*(1), Article 16457. https://doi.org/10.1038/s41598-020-73612-0

Wickramasinghe, N. N., Ravensdale, J., Coorey, R., Chandry, S. P., & Dykes, G. A. (2019). The predominance of psychrotrophic pseudomonads on aerobically stored chilled red meat. *Comprehensive Reviews in Food Science and Food Safety, 18*(5), 1622–1635. https://doi.org/10.1111/1541-4337.12483

Wickramasinghe, N. N., Ravensdale, J., Coorey, R., Dykes, G. A., & Chandry, P. S. (2021). Transcriptional profiling of biofilms formed on chilled beef by psychrotrophic meat spoilage bacterium, *Pseudomonas fragi* 1793. *Biofilms, 3*, Article 100045. https://doi.org/10.1016/j.bioflm.2021.100045

Wickramasinghe, N. N., Ravensdale, J. T., Coorey, R., Dykes, G. A., & Scott Chandry, P. (2019). *In situ* characterisation of biofilms formed by psychrotrophic meat spoilage pseudomonads. *Biofouling, 35*(8), 840–855. https://doi.org/10.1080/08927014.2019.1669021

Winsor, G. L., Van Rossum, T., Lo, R., Khaira, B., Whiteside, M. D., Hancock, R. E., & Brinkman, F. S. (2009). Pseudomonas genome database: Facilitating user-friendly, comprehensive comparisons of microbial genomes. *Nucleic Acids Research, 37*(Suppl. 1), D483–D488. https://doi.org/10.1093/nar/gkn861

Wu, Y., Ma, F., Pang, X., Chen, Y., Niu, A., Tan, S., Chen, X., Qiu, W., & Wang, G. (2022). Involvement of AprD in regulating biofilm structure, matrix secretion, and cell metabolism of meat-borne *Pseudomonas fragi* during chilled storage. *Food Research International, 157*, Article 111400. https://doi.org/10.1016/j.foodres.2022.111400

Yang, L., Hu, Y., Liu, Y., Zhang, J., Ulstrup, J., & Molin, S. (2011). Distinct roles of extracellular polymeric substances in *Pseudomonas aeruginosa* biofilm development. *Environmental Microbiology, 13*(7), 1705–1717. https://doi.org/10.1111/j.1462-2920.2011.02503.x

Yang, L., Liu, Y., Markussen, T., Høiby, N., Tolker-Nielsen, T., & Molin, S. (2011). Pattern differentiation in co-culture biofilms formed by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *FEMS Immunology and Medical Microbiology, 62*(3), 339–347. https://doi.org/10.1111/j.1574-695X.2011.00820.x

Ye, Z., Silva, D. M., Traini, D., Young, P., Cheng, S., & Ong, H. X. (2022). An adaptable microreactor to investigate the influence of interfaces on *Pseudomonas aeruginosa* biofilm growth. *Applied Microbiology and Biotechnology, 106*(3), 1067–1077. https://doi.org/10.1007/s00253-021-11746-5

Yin, R., Cheng, J., Wang, J., Li, P., & Lin, J. (2022). Treatment of *Pseudomonas aeruginosa* infectious biofilms: Challenges and strategies. *Frontiers in Microbiology, 13*. https://doi.org/10.3389/fmicb.2022.955286

Yu, H., Liu, Y., Li, L., Guo, Y., Xie, Y., Cheng, Y., & Yao, W. (2020). Ultrasound-involved emerging strategies for controlling foodborne microbial biofilms. *Trends in Food Science & Technology, 96*, 91–101. https://doi.org/10.1016/j.tifs.2019.12.010

Yuan, L., Liu, Y., Mi, Z., Xiong, D., Zhou, W., Xu, Z., Yang, Z., & Jiao, X. (2025). Dual-species biofilm and other profiles altered by interactions between *Salmonella Typhimurium* and *Pseudomonas fluorescens* isolated from meat. *Food Research International, 203*, Article 115914. https://doi.org/10.1016/j.foodres.2025.115914

Yuan, L., Sadiq, F. A., Wang, N., Yang, Z., & He, G. (2021). Recent advances in understanding the control of disinfectant-resistant biofilms by hurdle technology in the food industry. *Critical Reviews in Food Science and Nutrition, 61*(22), 3876–3891. https://doi.org/10.1080/10408398.2020.1809345

Yuan, L., Zhang, Y., Mi, Z., Zheng, X., Wang, S., Li, H., & Yang, Z. (2024). Calcium-mediated modulation of *Pseudomonas fluorescens* biofilm formation. *Journal of Dairy Science, 107*(4), 1950–1966. https://doi.org/10.3168/jds.2023-23860

Zeng, G., Vad, B. S., Dueholm, M. S., Christiansen, G., Nilsson, M., Tolker-Nielsen, T., Nielsen, P. H., Meyer, R. L., & Otzen, D. E. (2015). Functional bacterial amyloid increases *Pseudomonas* biofilm hydrophobicity and stiffness. *Frontiers in Microbiology, 6*. https://doi.org/10.3389/fmicb.2015.01099

Zhang, Y., Silva, D. M., Young, P., Traini, D., Li, M., Ong, H. X., & Cheng, S. (2022). Understanding the effects of aerodynamic and hydrodynamic shear forces on *Pseudomonas aeruginosa* biofilm growth. *Biotechnology and Bioengineering, 119*, 1483–1497. https://doi.org/10.1002/bit.28077

Zhou, G., Dong, P., Luo, X., Zhu, L., Mao, Y., Liu, Y., & Zhang, Y. (2024). Combined effects of cold and acid on dual-species biofilms of *Pseudomonas fluorescens and Listeria monocytogenes* under simulated chilled beef processing conditions. *Food Microbiology, 117*, Article 104394. https://doi.org/10.1016/j.fm.2023.104394

Zhou, G., Liu, Y., Dong, P., Mao, Y., Zhu, L., Luo, X., & Zhang, Y. (2024). Airborne signals of *Pseudomonas fluorescens* modulate swimming motility and biofilm formation of Listeria monocytogenes in a contactless coculture system. *Food Microbiology, 120*, Article 104494. https://doi.org/10.1016/j.fm.2024.104494

Zhu, Y., Li, C., Cui, H., & Lin, L. (2020). Feasibility of cold plasma for the control of biofilms in food industry. *Trends in Food Science & Technology, 99*, 142–151. https://doi.org/10.1016/j.tifs.2020.03.001