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Review of *Pseudomonas* Attachment and Biofilm Formation in Food Industry

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Abstract

During the last few decades, extensive attention has been paid on the management of food alteration and contamination caused by spoilage organisms such as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. The ability to metabolize a variety of diverse nutrients enables these bacteria to survive in a variety of habitats by developing biofilms. Biofilms are more resistant to antimicrobials making their elimination from food processing facilities a big challenge. However, understanding the mechanisms by which these microorganisms develop biofilm has remained elusive. In this background and because of their resistant phenotype, attention should be focused on better understanding how to eradicate completely *Pseudomonas* biofilm. In this review our ultimate goal is to covers recent advances in biofilm formation and to describe certain factors linked to biofilm resistance. It is important to note that future endeavors are needed to elucidate mechanisms that reduce the susceptibility of biofilms to biocides.

Keywords: Management; *Pseudomonas*; Biofilms; Food processing

Introduction

Within biofilms microorganisms are generally well-protected against the influence of disinfectants, antibiotics [1] and the host immune system [2], and as a consequence biofilms are extremely difficult to eradicate [3]. As such, they cause major problems in medicine, agriculture, (food) industry and the household environment. Biofilms are problematic in particular food industry sectors such as brewing, dairy processing, fresh produce, poultry processing and red meat processing [4,5]. Reduction of bacterial contamination of poultry products during processing is of major concern among processors and those concerned with food safety. Biofilm can grow on abiotic surfaces of different equipment in food industry and poses a risk to human health. Furthermore, food processing surfaces and equipment may be favorable environments for *Pseudomonas* biofilm formation. This is why many potential *Pseudomonas* species virulence factors have been identified using animal models of infection, including those that allow bacteria to move over, attach to, and effectively colonize mammalian tissues, and secreted enzymes and toxins that allow *Pseudomonas* to cross tissue barriers and evade host immune defences. In this background, attention should be focused on better understanding the factors influencing production and distribution of the biofilm in certain species of *Pseudomonas* which had a rapid biofilm evolution, adaptation and resistance.

An initial step in biofilm formation is bacterial attachment to a surface depends on two surface organelles, flagella and type IV pili (tfp). Bacteria lacking flagella caused less inflammation [6], and are required for robust biofilm formation [7,8], and likely contribute to persistent colonization. Type IV pili, which power twitching motility across solid surfaces [9], mediate attachment to epithelial cells, and contribute to biofilm formation [10]. Gram-negative bacteria, including *Pseudomonas* were isolated from samples of steel, rubber, and cast iron chips positioned on floor drains and food contact surfaces in several food-processing environments [11]. It should be noted that comparative studies between attached bacteria and planktonic bacteria (those not attached) showed that when microorganisms attached to surfaces, they became more resistant to the chemicals [12]. It is also interesting to note that research on the physical and chemical characteristics of bacterial attachment presents the opportunity for reduction of pathogens and spoilage organisms by preventing the formation or buildup of biofilms.

Food safety could be enhanced by increasing the use of materials that prevent attachment of microorganisms prone to develop into biofilms while decreasing the use of materials that encourage bacterial attachment. Prevention of biofilm development could also reduce the use of chemicals in food plant sanitation, thereby lowering the costs to produce a safe, whole-some product [12].

This review covers recent advances in biofilm formation and introduces the phenomenon of swarming motility from a practical standpoint, then describes certain factors linked to biofilm resistance.

The presence of *Pseudomonas* biofilms in food production facilities

Biofilms have been found not only on food production surfaces but on food products themselves, including sprouts, spinach, and lettuce [13]. They have been also associated with environmental surfaces, such as floors, walls, pipes, drains and seem to prefer certain food contact surface which provides the necessary nutrients and optimal growth condition. These areas often are hard to reach during cleaning and sanitation and thus optimal conditions for the formation and development of biofilms are established. Food-processing environments provide a variety of conditions, which might favor the formation of biofilm, for instance: presence of moisture, nutrients and inocula of micro-organisms from the raw materials [14]. Such a biofilm is a potential source of contamination of foods that may lead to spoilage or transmission of foodborne pathogens [15]. Furthermore, the occurrence of foodborne disease outbreaks has been increasing considerably every year despite the continuous advances in food processing, hygiene, and cleaning and sanitizing procedures.

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Currently, food safety has been the greatest concern of consumers, food industry, and health public authorities. Making it difficult to meet hygiene standards, as the adhered cells are more resistant to detergents and sanitizing actions [16].

It has long been recognized that equipment and utensil surfaces can help microorganisms adhere, grow, and become a source of spoilage and pathogenic microorganisms such as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* which are members of the spoilage bacteria and are also involved in adherence processes and biofilm formation. These both spoilage organisms' are found in food processing environments including drains and floors, on fruits, vegetables, meat surfaces.

Pseudomonas sp. are one of the most important bacteria causing spoilage of conventionally pasteurized liquid milk products, acting by two different routes. First, they produce the majority of lipolytic and proteolytic enzymes secreted into raw milk during pre-processing storage, even in psychrotropic environments. Many of these enzymes can survive pasteurization and even ultra-high-temperature treatments and can thus reduce the sensory quality and shelf life of the processed liquid milk products. Second, *Pseudomonas* sp. can act in the post-pasteurization process, causing spoilage of conventionally pasteurized milk during refrigerated storage [17].

Importance of *Pseudomonas* attachment

The extracellular filamentous appendages produced by motile microorganisms are responsible for the attachment process and interact with surface in different manner. Till date, Flagella, and pili had been the subject of intense study mainly for two reasons. First, their responsibility in behavior motility. Second, because of their consideration as one of the three major matrix components (as presented below).

Flagella are very fine threads of the protein flagellin with a helical structure extending out from the cytoplasm through the cell wall. Flagella may have a diameter between 0.01 and 0.02 mm, and a length of up to 10 mm. Many types of bacteria have flagella, including the genus *Pseudomonas*. It is possible that the flagellum itself may form an adhesive bond with the adhesion surface [18]. The primary function of flagella in biofilm formation is assumed to be in transport and in initial cell–surface interactions [19]. Flagella-mediated motility is believed to overcome repulsive forces at the surface of the substratum. Moreover, pili or fimbriae are found on many Gram-negative bacteria including *Pseudomonas* species. They are fine, filamentous appendages, also of protein, 4-35 nm wide and up to several micrometers long [18]. These structures are usually straight, and are not involved in motility. Their only known general function is to make cells more adhesive, since bacteria with pili can adhere strongly to other bacterial cells and inorganic particles.

Biofilm development involves several stages which must be understood in order to achieve biofilm control and which begin by the attachment of pioneer bacteria to the surface. Biofilm formation and swarming motility are inversely regulated. This phenotype requires coordination of several pathways, including flagellar motility, cell-cell signaling/quorum sensing and bio-surfactant secretion [20]. Swarming motility is operationally defined as a rapid multicellular movement of bacteria across a surface, powered by rotating flagella [21]. Swimming motility is a mode of bacterial movement that is also powered by rotating flagella but, unlike swarming motility, swimming takes place as individual cells moving in liquid environments. Twitching motility is a surface motility powered by the extension and retraction of type IV pili, which confers slow cell movement, often with a jerky or 'twitchy' appearance [22]. Gliding motility is a catch-all definition for active surface movement that occurs along the long axis of the cell without the aid of flagella or pili. Gliding seems to have evolved independently in multiple lineages but generally involves the cell body moving through the use of focal-adhesion complexes that bind to a surface substrate. Sliding motility is a passive form of surface spreading that does not require an active motor but instead relies on surfactants to reduce surface tension, enabling the colony to spread away from the origin, driven by the outward pressure of cell growth [21].

Biofilm-forming bacteria are generally known to employ both extracellular and intracellular biofilm factors including membrane appendages and extracellular matrices [20]. As is the case with most bacteria, environmental isolates of *Pseudomonas* are capable of forming different types of biofilm, including pellicles (floating biofilms at the air liquid interface) or wrinkly spreaders (WSs, or solid surface-associated submerged biofilms) [21]. Recent studies involving *Pseudomonas fluorescens* WSs have shown that certain factors including cellulose matrix, fimbriae, and lipopolysaccharides (LPS) might be extremely relevant to the strength and integrity of WS [9].

Overview of biofilms development

As presented in precedent sections some bacterial pathogens exhibit multicellular behaviors as a conserved strategy for long-term bacterial survival in nature and during infections. One of these multicellular behaviors is biofilm formation [22] which represents a protective mode of growth that allows microorganisms to survive in hostile environments and disperse seeding cells to colonize new niches under desirable conditions [23].

Biofilms are intricate bacterial communities found attached to living or abiotic surfaces and surrounded by a bacterially-produced extracellular matrix composed of exopolysaccharides, DNA and proteins [24]. Biofilms develop in a complex and well-coordinated manner that involves sensing and responding to cues, such as bacterial cell density, nutrient availability and energy sources present in the environment. The switch toward the biofilm mode of growth is often considered to be a survival strategy for bacteria [25].

Several factors are required for biofilm development, including attachment via adhesive protein, cell aggregation via proteins, extracellular DNA, polysaccharides, and cell motility [26]. Furthermore, biofilm development (Figure 1) occurs via a series of well-defined stages which include; (i) translocation to the surface and initial and reversible attachment of cells, (ii) irreversible attachment, (iii) microcolony formation, (iv) maturation and differentiation of the biofilm, and (v) dispersal of single cells from the biofilm [27]. Biofilm formation has been linked to the survival of pathogenic bacteria and it has been connected to infections associated with indwelling medical devices. Indeed, biofilm bacterial communities confer a protection from environmental hazards [28].

The surface colonization can take place either at the solid-liquid interface (SLI-biofilm) or at the air-liquid interface (ALI-biofilm) where it forms a pellicle on the top of the liquid media as observed in *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* [29].

Biofilm architectures and structure

Biofilm architectures are highly variable, ranging from open structures containing channels and columns of bacteria, to structures

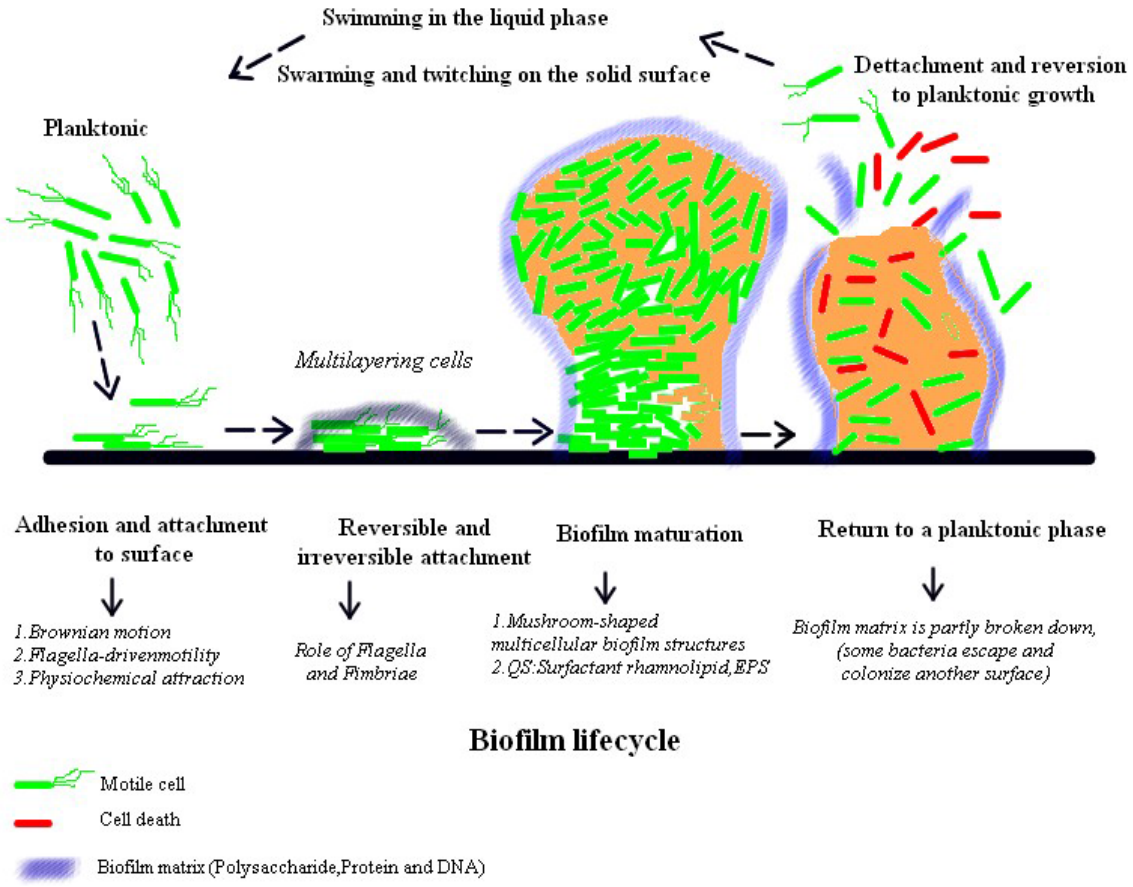

Figure 1: Biofilm lifecycle occurs in several stages, comprising the initial attachment where bacteria adhere via Brownian motion, flagella-driven motility and physiochemical attraction (van der Waals interactions). During stage 2-3, reversible and irreversible attachment, Flagella and Fimbriae permanently anchor the bacteria to the surface. During stage 4, biofilm maturation through cell division and an extracellular matrix composed primarily of polysaccharides holds the biofilm together. During stage 5, dispersal, the biofilm matrix is partly broken down and returns to planktonic phase, some bacteria escape to colonize another surface.

with no obvious pores and densely packed regions of cells [30]. The close physical association of cells within these biofilms results in a structure with significant physical properties and changes in bacterial physiology compared with free-living cells [31].

The complex structure of microbial biofilms has only recently been determined. Detailed analysis by scanning confocal laser microscopy has shown that biofilms of *P. aeruginosa* formed on solid surfaces and exposed to a continuous flow of fresh nutrients are open, highly hydrated structures consisting of cells embedded in an extracellular matrix filled with large void spaces [32]. The ability to form biofilm is a universal attribute of several bacteria but the mechanism that different bacterial species employ to produce biofilm may vary according to the specific strain attributes and the diverse environments they occupy.

Even though some biofilm structural components can be recognized as common features, their chemical compositions may vary. The process of biofilm formation is dynamic and complex but the stages of development seem to be conserved among a remarkable range of prokaryotes and typically involve the attachment to a surface by planktonic bacteria, replication, cell-cell adhesion to form microcolonies, maturation, and detachment [33] (as shown in Figure 1).

Global regulating systems involved in biofilm formation

The successful adaptation of bacteria to changing natural conditions is dependent on their ability to sense and respond to the external environment and modulate gene expression accordingly [34]. Based on auto-induction process, quorum sensing provides a mechanism for self-organization and regulation of microbial cells [35] in which an environmental sensing system allows bacteria to monitor and respond to their own population densities. Quorum sensing systems are known to be involved in a range of important microbial activities. These include extracellular enzyme biosynthesis, biofilm development, antibiotic biosynthesis, bio-surfactant production, EPS synthesis and extracellular virulence factors in Gram-negative bacteria [34]

As summarized in Figure 2, *P. aeruginosa* produces a diffusible organic signal, originally called an auto-inducer (AI) molecules (Oligopeptides, N-acylhomoserine lactones (AHL), which accumulates in the surrounding environment during growth [36]. This QS systems control the lifestyles of various pseudomonads, enabling them to successfully colonize a wide variety of ecological niches.

Furthermore, the complex regulation of biofilm formation involves multiple bacterial machineries, including the QS systems and the two-component regulatory systems that both interact mainly with EPS production.

As a cell-to-cell communication, this system is used by many bacteria to detect their population density by producing and perceiving diffusible signal molecules that coordinate virulence factors production,

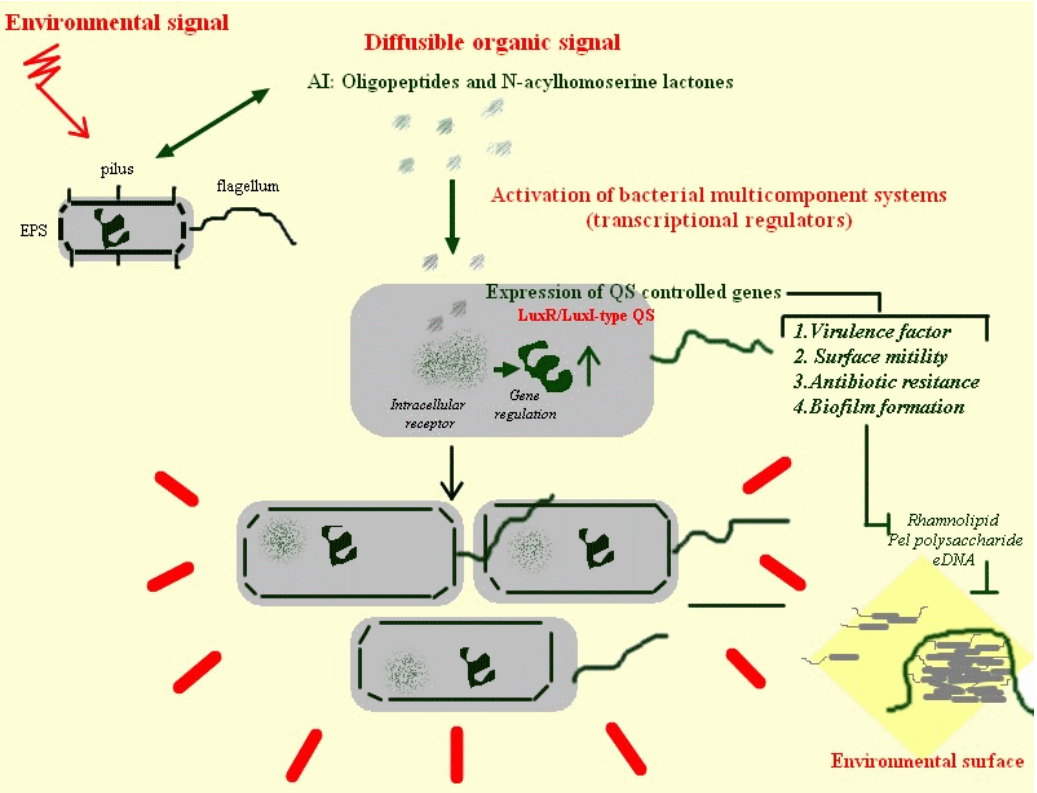

Figure 2: Vis a vis an environmental signal, bacteria like the *P. aeruginosa* responds and produces an auto-inducer (AI) molecules (N-acylhomoserine lactones (AHL) which control the lifestyles of various pseudomonads, enabling them to successfully colonize surfaces by biofilm formation.

motility, and biofilm formation [37]. In the case of *P. aeruginosa*, two main QS systems (las and rhl) which drive the production (throughout synthases LasI and RhlI) and the perception (by the transcription factors LasR and RhlR) of the autoinducer signaling molecules N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) and N-butanoyl-L-homoserine lactone (C4-HSL), respectively. A third QS system, based on quinolone signals (PQS system), interacts with the acyl homoserine lactones (AHLs) systems in an intricate way [38].

Thus, numerous post-transcriptional regulatory factors, mainly affecting biofilm maturation at the level of synthesis or exopolysaccharides has been described currently. Moreover, environmental factors such as nutrient concentrations (glucose, amino acids) and other physiological stresses (osmolarity, pH, oxidative stress, antimicrobials) are important signals mediating the switch from the planktonic motile to sessile biofilm lifestyles [33].

Extracellular matrix

The cell–cell interconnecting network in biofilms is usually referred to as the extracellular biofilm matrix and is composed of a variety of biopolymers, including polysaccharide, protein and DNA. A single bacterial species can produce several different biofilm matrix components and it appears that not all of these components are expressed during biofilm formation in a particular environment [27].

The EPS of biofilm is a mixture of polysaccharides, extracellular DNA (eDNA), and proteins, which function as matrix, or glue, holding microbial cells together [23]. Exopolysaccharides (EPSs) are the major components of bacterial biofilm matrix. They are secreted by microorganisms and play an essential role in biofilm structure and in microbial activity. They mediate the transport of chemicals to and from the microorganisms. Moreover, they also show ion exchange properties due to negatively charged surface functional groups, which bind to cationic species. EPS matrix also provides an effective barrier that restricts penetration of chemically reactive biocides, antibiotics and antimicrobial agents. It has been shown that bacteria living in biofilm matrix can be up to 1000 times more resistant to antibacterial compounds than planktonic bacteria [39].

A general hallmark feature that determines the mature biofilm architecture is the presence of the extracellular matrix (EM) surrounding the resident biofilm bacteria [33]. The biofilm matrix generally consists of up to 97% water, 2–5% microbial cells, 3–6% extra-polymeric substances (EPS) and ions [40]. The EPS may account for 50–90% of the total organic carbon of biofilm and this is primarily composed of exopolysaccharides, but it also includes proteins (extracellular proteins and enzymes), DNA and RNA, which constitute less than 2% of the biofilm matrix [41]. The polysaccharide composition along with other components such as proteins usually varies among different bacteria and even between strains of a single species, although there are some common polysaccharides produced by multiple species of bacteria. The most famous exopolysaccharides present inside biofilms are alginate, cellulose and poly-N-acetyl glucosamine. However, *P. aeruginosa* can produce at least three different exopolysaccharides: alginate, PEL and PSL.

Alginates, which are overproduced by *P. aeruginosa* after infection of CF patients, are linear polyanionic exopolysaccharides composed of uronic acids [42]. Alginate has been shown to contribute to decreased susceptibility of biofilms to antibiotic treatment and human

antibacterial defense mechanisms [43]. The Psl polysaccharide is rich in mannose and galactose and is involved in initial attachment and mature biofilm formation. Psl is produced during planktonic growth, mediating attachment to surfaces and contributing to micro colony formation. In mature biofilms, Psl is associated with the caps of mushroom-like micro colonies, forming a peripheral meshwork covering the cap region [44].

Pel is a glucose-rich, cellulose-like polymer essential for the formation of a pellicle at the air-liquid interface. Increased Pel production has also been associated with the wrinkled colony phenotype. It has recently been shown that Pel plays a role in cell-to-cell interactions in *P. aeruginosa* PA14 biofilms, providing a structural scaffold for the community at early stages of biofilm formation [45].

To date, the exopolysaccharide matrix has not been directly visualized at distinct developmental stages during *P. aeruginosa* biofilm formation. It has been proposed that after contact of bacteria with a surface, altered gene expression induces changes that initiate synthesis of extracellular polysaccharides since alginate, the EPS of *P. aeruginosa* biofilms, is up-regulated in recently attached cells in comparison with planktonic cells [34].

Extracellular DNA (eDNA)

Besides the important role of the exopolysaccharides in biofilm formation, extracellular DNA (eDNA) has been shown to be an important component of the biofilm matrix. Moreover, eDNA was found to mediate cell-cell interactions in biofilms [39]. In addition the eDNA appears to be derived from random chromosomal DNA, which functions as a cell-to-cell inter-connecting component in the biofilm. Cells also undergo autolysis in biofilm microcolonies [45], but it is unclear whether autolysis contributes to eDNA and biofilm development. It has also been shown that eDNA in the biofilm matrix contributes to cation gradients, genomic DNA release and inducible antibiotic resistance [46,47].

In *P. aeruginosa*, eDNA release is mediated through quorum sensing (QS) dependent mechanisms, involving N-acyl-L-homoserine lactones (AHL) and the *Pseudomonas* quinolone signaling (PQS) molecule, and through QS independent mechanisms via flagella and type IV pili . PQS in *P. aeruginosa* PAO1 triggers eDNA release in the early phase of planktonic culture through induction of prophage [39].

Pili, flagella, and fimbriae

Aside from both components, extracellular proteins and several proteinaceous components are also considered to be matrix components, including type IV pili, flagella, and fimbriae. These components were found to mainly play auxiliary functions as adhesion factors and structural support in the biofilm formation of *P. aeruginosa* [24]. Flagella mediate swimming and swarming motility of *P. aeruginosa* (Figure 3) (as reported in section 3). It can also act as an adhesin and play critical roles in the initial cell-to-surface interactions [10]. The successful adhesion of Gram-negative bacteria to surfaces is largely dependent on the presence of cell appendages such as flagella, pili, and fimbriae [48]. Furthermore, the presence of functional flagella enables the bacterium to swim and overcome repulsive electrostatic forces that may exist between the cell surface and the surface of material or the host's conditioning film [10].

Type IV pili (T4P) is a linear actuator critical for twitching motility that involves an extension-grip-retraction mechanism. T4P plays important roles in microcolony formation of *P. aeruginosa* biofilms by forming typical mushroom caps [10].

The *P. aeruginosa* cup fimbriae constitute one class of appendages that facilitate the biofilm formation and assemble through chaperone/usher pathway. It was demonstrated that Cup fimbriae are critical for the initial stage of biofilm development, particularly in cell-to-cell interaction and micro colony formation [49].

Rhamnolipid biosurfactants as new players in rhamnolipids and biofilms

Rhamnolipids (RLs) are glycolipid biosurfactants produced by various bacterial species including some *Pseudomonas* sp. and *Burkholderia* sp. [50]. The RLs are amphiphilic molecules highly diverse and those produced by *Pseudomonas aeruginosa* have been extensively studied. Because of their excellent surface activity and hydrocarbon-solubilizing properties, the physicochemical properties of RLs have received considerable interest [51] and they also have been used in the fields of bioremediation and biodegradation [52]. It is noteworthy that rhamnolipids are essential to maintain the architecture of the biofilms and are considered as one of the virulence factors in *Pseudomonas* sp. [53] and required for *P. aeruginosa* swarming patterns of cells organized as radiating tendrils (Figure 3).

In this context of food safty and in addition to the treatment of biomaterials used for medical devices, biosurfactants have also been used in the pretreatment of material surfaces found in food-processing environments.

Pathogenic bacteria implicated in food-borne illness outbreaks are able to form biofilms on food contact surfaces that are more resistant to sanitation than free-living cells [54]. The pre-conditioning of surfaces using microbial surface-active compounds may be an interesting strategy for preventing the adhesion of food-borne pathogens to solid surfaces. As was pointed out by Meylheuc et al. [55] in which the preconditioning of stainless steel surfaces with an anionic biosurfactant produced by *P. fluorescens* had reduced the number of *L. monocytogenes* LO28-adhering cells and thus favoured the bactericidal activities of the disinfectants sodium hypochlorite (NaOCl) and peracetic acid/hydrogen peroxide (PAH).

Similarly, biosurfactants obtained from *P. fluorescens* were able to inhibit the adhesion of four *Listeria* strains to stainless steel [55]. More recently, another group investigated the effect of rhamnolipid and surfactin biosurfactants on the adhesion of the food pathogens *E. sakazakii*, *L. monocytogenes* and *S. enteritidis* to polypropylene and stainless steel surfaces [56].

Nowadays, activity demonstrated by biosurfactants suggests that they could be considered as new tools in developing strategies to prevent or delay microbiological colonization of industrial plant surfaces used in foodstuffs preparation [57]. Furthermore, these tensoactive compounds have biocide properties, are applicable in bioremediation processes and their ability to modify the surfaces of cells and materials have been studied. RLs have also been proposed to be used in food industry applications [57]. *P. putida* biosurfactant have a direct biocide action on *P. aeruginosa* and was able to avoid their biofilm formation in surfaces of polyvinyl chloride [58]. Rhamnolipids also can be effective to disrupt biofilms. Irie et al. [59] describe that rhamnolipids disperse biofilm of *Bordetella bronchiseptica*, whereas Dusane et al. [60] reported that rhamnolipids were also effective as an anti-adhesive and biofilm disrupting agent against *Bacillus pumilus*.

Interestingly, deep insight into the biological importance of *Pseudomonas* RLs would reveal new approach in food safety and public health, and will certainly provide fascinating aspects of beneficial use.

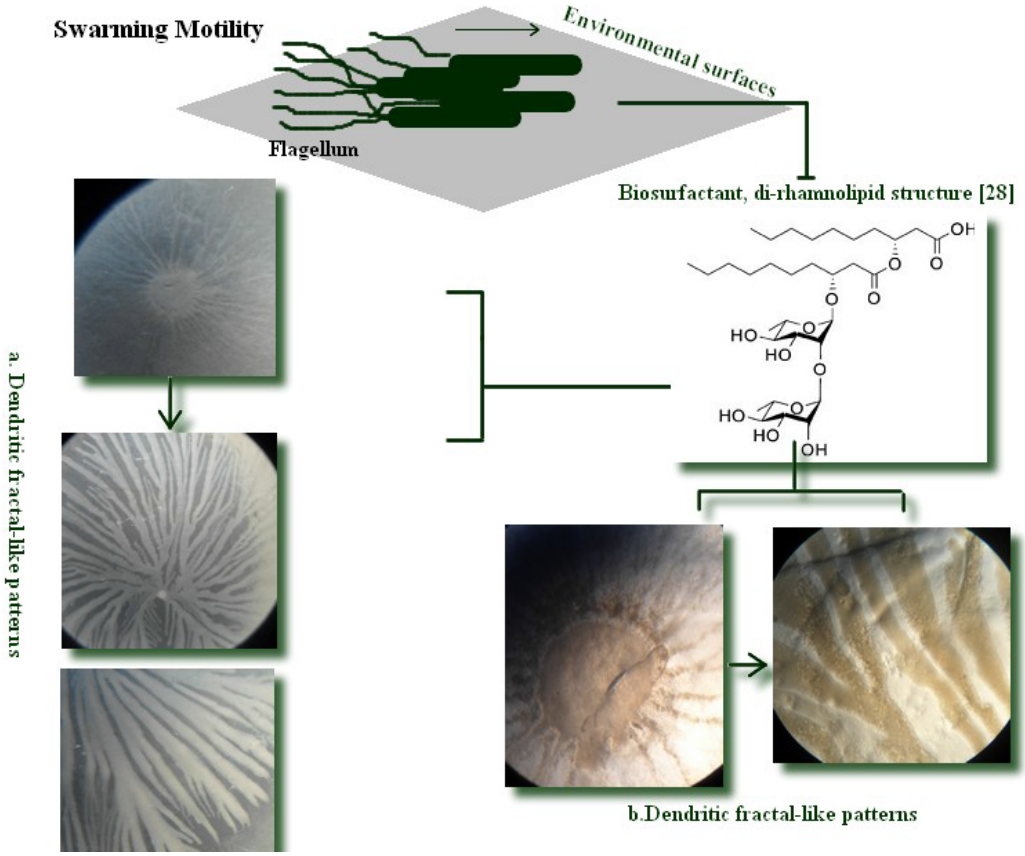

Figure 3: Rhamnolipids are required for modulating swarming behavior in different certain species of Pseudomonas. Note the formation of dendritic fractal-like patterns formed by migrating swarms moving away from an initial location these species. Swarming motility on TSA medium (1.5% agar) for 72 h at 30°C after a central spot of 5 ml of an overnight bacterial culture in TSB. Views of swarming motility are provided from my personal current study.

Conclusions

In food industry sectors, biofilms may be a source of recalcitrant contaminations, causing food spoilage and generating public health problems. In the perspective of food safety, many studies have been progressed for understanding *Pseudomonas* attachment and biofilm development in different environmental niches. To the best of our knowledge, reduction of *Pseudomonas* species contamination of food products during processing or during food conservation is of major concern and requires a complete identification of bacterial and ecological factors, which influence the establishment and virulence of biofilms. Understanding these factors helps us to identify novel targets for biofilm interventions and, thereby, enhance plant sanitation and pathogen control.

References

1. Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O (2010) Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 35: 322 - 332.
2. Jensen PO, Givskov M, Bjarnsholt T, Moser C (2010) The immune system vs. *Pseudomonas aeruginosa* biofilms. FEMS Immunol Med Microbiol 59: 292- 305.
3. Burmolle M, Thomsen TR, Fazli M, Dige I, Christensen L, et al. (2010) Biofilms in chronic infections-A matter of opportunity monospecies biofilms in multispecies infections FEMS Immunol. Med Microbiol 59: 324-336.
4. Chen J, Rossman ML, Pawar DM (2007) Attachment of enterohemorragic Escherichia colito the surface of beef and a culture medium. LWT-Food Science and Technology 40: 249 - 254.
5. Jessen B, Lammert L (2003) Biofilm and disinfection in meat processing plants. International Biodeterioration and Biodegradation 51: 265-269.
6. Feldman M, Bryan R, Rajan S, Scheffler L, Brunnert S, et al. (1998) Role of flagella in pathogenesis of Pseudomonas aeruginosapulmonary infection. Infect Immun 66: 43-51
7. Balloy V, Verma A, Kuravi S, Si-Tahar M, Chignard M, et al. (2007) The role of flagellin versus motility in acute lung disease caused by *Pseudomonas aeruginosa*. J Infect Dis 196: 289-296.
8. Klausen M, Heydorn A, Ragas P, Lambertsen L, Aaes-Jorgensen A, et al. (2003) Biofilm formation by Pseudomonas aeruginosawild type, flagella and type IV pili mutants. Mol Microbiol 48: 1511-1524.
9. Mattick JS (2002) Type IV pili and twitching motility. Annu Rev Microbiol 56: 289-314.
10. O'Toole GA, Kolter R (1998) Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol Microbiol 30: 295-304.
11. Zottola EA (1994) Scientific status summary "Microbial attachment and biofilm formation: a new problem for the food industry?" Food Technol 48:107-114
12. Joseph B, Otta SK, Karunasagar I, Karunasagar I (2001) Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. Intl J Food Microbiol 64: 367-372.
13. Fett W (2000) Naturally occurring biofilms on alfalfa and other types of sprouts. J Food Prot 63: 625-632.
14. Bower CK, McGuire J, Daeschel MA (1996) The adhesion and detachment of bacteria and spores on food-contact surfaces. Trends Food Sci Technol 7: 152-157.

15. Gunduz GT, Tuncel G (2006) Biofilm formation in an ice cream plant. Antonie van Leeuwenhoek 89: 329-336.

16. Wirtanen G, Salo S (2005) Biofioms risks. In: Lilieveld, H. L. M.; Mostert, M. A.; Holah, J. Handbook of hygiene control in the food industry. Cambrige, England: CRC Press, 46-62.

17. Dogan B, Boor KJ (2003) Genetic diversity and spoilage potential among *Pseudomonas* spp. isolated from fluid milk products and dairy processing plants. Applied and Environmental Microbiology 69: 130-138.

18. Harbron RS, Kent CA (1988) Aspects of cell adhesion. In Melo L F, Bott TR, and Bernardo CA (Eds), .NATO ASI series 145: 125-140.

19. Sauer K, Camper AK (2001) Characterization of phenotypic changes in *Pseudomonas putida* in response to surface-associated growth. Journal of Bacteriology183: 6579-6589.

20. De´ziel E, Le´pine F, Milot S, Villemur R (2003) rhlA is required for the production of a novel biosurfactant promoting swarming motility in *Pseudomonas aeruginosa*: 3-(3-hydroxyalkanoyloxy) alkanoic acids (HAAs), the precursors of rhamnolipids. Microbiology 149: 2005-2013.

21. Kearns Daniel B (2010) A field guide to bacterial swarming motility. Nature Reviews Micro Biology 8: 634-644.

22. Mah TF, O'Toole GA (2001) Mechanisms of biofilm resistance to antimi-crobial agents. Trends Microbiol 9: 34-39.

23. Qing Wei, Luyan Z Ma (2013) Biofilm Matrix and Its Regulation in Pseudomonas aeruginosa. Int J Mol Sci 14: 20983-21005.

24. Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS (2002) Extra-cellular DNA required for bacterial biofilm formation. Science 295: 1487.

25. Ho ¨iby N, Krogh Johansen H, Moser C, Song Z, et al. (2001) *Pseudomonas aeruginosa* and thein vitro andin vivo biofilm mode of growth. Microbes Infect 3:23-35.

26. Kjelleberg S, Givskov M (2007) The Biofilm Mode of Life: Mechanisms and Adaptations (eds. S. Kjelleberg and M. Givskov), Horizon Bioscience, Wymondham, 5-21.

27. Rehm Bernd HA (2008) *Pseudomonas*. Model Organism, Pathogen, Cell Factory. Edited by WILEY-VCH Verlag GmbH and Co. KGaA: 144.

28. Davey ME, O'Toole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64: 847-867.

29. Meliani A, Bensoltane A (2014) Enhancement of Hydrocarbons Degradation by Use of Pseudomonas Biosurfactants and Biofilms. J Pet Environ Biotechnol 5:168.

30. Watnick P, Kolter R (2000) Biofilm, City of microbes. J Bacteriol. 182: 2675-2679.

31. Kuchma SL, O'Toole GA (2000) Surface-induced and biofilm induced changes in gene expression. Curr Opin Biotechnol 11: 429-433.

32. Lawrence JR, Korber DR, Hoyle BD, Costerton JW, Caldwell DE (1991) Optical sectioning of microbial biofilms. J Bacteriol 173: 6558-6567.

33. Martínez LC, Vadyvaloo V (2014) Mechanisms of post-transcriptional gene regulation in bacterial biofilms. Front. Cell Infect Microbiol 4: 38.

34. Daniels R, Vanderleyden J, Michiels J (2004) Quorum sensing and swarming migration in bacteria. FEMS Microbiology Reviews 28: 261-289.

35. Parsek MR, Greenberg EP (2005) Sociomicrobiology: the connections between quorum sensing and biofilms.Trends in Microbiology 13: 27-33.

36. Fuqua C, Greenberg EP (2002) Listening in on bacteria acyl-homoserine lactone signalling. Nature Reviews in Molecular Cell Biology 3: 685-695.

37. Jimenez PN, Koch G, Thompson JA, Xavier KB, Cool RH, Quax WJ (2012) The multiple signaling systems regulating virulence in *Pseudomonas aeruginosa*. Microbiology and Molecular Biology Reviews 76: 46-65.

38. De Kievit TR (2009) Quorum sensing in *Pseudomonas aeruginosa* biofilms. Environmental Microbiology 11: 279-288.

39. Remminghorst U, Hay ID, Rehm B H (2009) Molecular characterization of Alg8, a putative glycosyltransferase, involved in alginate polymerisation. J Biotechnol 140: 176-183.

40. Sutherland IW (2001) The biofilm matrix–an immobilized but dynamic microbial environment.Trends Microbiol 9: 222-227.

41. Flemming HC, Neu TR, Wozniak DJ (2007) The EPS matrix: the "house of biofilm cells". J Bacteriol 189: 7945-7947.

42. Pier GB, Coleman F, Grout M, Franklin M, Ohman DE (2001) Role of alginate O acetylation in resistance of mucoid *Pseudomonas aeruginosa* to opsonic phagocytosis. Infect. Immun 69: 1895-1901.

43. Luyan Ma, Matthew Conover, Haiping Lu, Matthew R Parsek, Kenneth Bayles, et al. (2009) Assembly and development of the *Pseudomonas aeruginosa* biofilm matrix. PLoS Pathog 5: e1000354.

44. Ghafoor A, Hay Iain D, Bernd Rehm HA (2011) Role of Exopolysaccharides in Pseudomonas aeruginosa Biofilm Formation and Architecture. Appl Environ Microbiol 77: 5238-5246.

45. Allesen Holm M, Barken KB, Yang L, Klausen M, Webb JS, et al. (2006) A characterization of DNA release in Pseudomonas aeruginosa cultures and biofilms. Mol Microbiol 59: 1114-1128.

46. Webb JS, Thompson LS, James S, Charlton T, Tolker-Nielsen T, et al. (2003) Cell death in Pseudomonas aeruginosa biofilm development. J Bacteriol 185: 4585- 4592.

47. Mulcahy H, Charron-Mazenod L, Lewenza S (2008) Extracellular DNA Chelates Cations and Induces Antibiotic Resistance in Pseudomonas aeruginosa Biofilms. PLoS Pathog 4: e1000213.

48. Lejeune P (2003) Contamination of Abiotic Surfaces: What a Colonizing Bacterium Sees and how to Blur it. Trends Microbiol 11: 179-184.

49. Kulasekara HD, Ventre I, Kulasekara BR, Lazdunski A, Filloux A, et al. (2005) A novel two-component system controls the expression of *Pseudomonas aeruginosa* fimbrial cup genes. Mol Microbiol 55: 368-380.

50. Abdel-Mawgoud AM, Lepine F, Deziel E (2010) Rhamnolipids: Diversity of structures, microbial origins and roles. Appl Microbiol Biotechnol 86: 1323-1336.

51. Abdel-Mawgoud AM, Aboulwafa MM, Hassouna NAH (2009) Characterization of rhamnolipid produced byPseudomonas aeruginosa isolate BS20. Appl Biochem Biotechnol 157: 329-345.

52. Cameotra SS, Singh P (2009) Synthesis of rhamnolipid biosurfactant and mode of hexadecane uptake by Pseudomonas species. Microb Cell Fact 8: 16.

53. Ron EZ, Rosenberg E (2001) Natural roles of biosurfactants. Environ Microbiol. 3: 229-236.

54. Stepanovic S, Cirkovic I, Ranin L, Svabic-Vlahovic M (2004) Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. Lett Appl Microbiol 38: 428-432.

55. Meylheuc T, Methivier C, Renault M, Herry JM, Pradier CM, et al. (2006) Adsorption on stainless steel surfaces of biosurfactants produced by gram-negative and gram-positive bacteria: consequence on the bioadhesive behavior of *Listeria monocytogenes*. Colloids Surf B Biointerfaces 52: 128-137.

56. Nitschke M, Araújo LV, Costa SG, Pires RC, Zeraik AE, et al. (2009) Surfactin reduces the adhesion of food-borne pathogenic bacteria to solid surfaces. Lett Appl Microbiol 49: 241-247.

57. Nitschke M, Costa SG, Contiero J (2010) Structure and applications of a rhamnolipid surfactant produced in soybean oil waste. Appl. Biochem. Biotechnol 160: 2066-2074.

58. Al-Tahhan RA, Sandrin TR, Bodour AA, Maier RM (2000) Rhamnolipid-induced removal of lipopolysaccharide from *Pseudomonas aeruginosa*: Effect on cell surface properties and interaction with hydrophobic substrates. Appl Environ Microbiol 66: 3262-3268.

59. Irie Y, O`Toole GA, Yuk MH (2005) *Pseudomonas aeruginosa* rhamnolipids disperse *Bordetella bronchiseptica* biofilms. FEMS Microbiology Letters 250: 237-243.

60. Duzane DH, Nancharaiah V, Zinjarde SS, Venugopalan VP (2010) Rhamnolipid mediated disruption of marine *Bacillus pumilus* biofilms. Colloids and Surfaces B: Biointerfaces 81: 242-248.