Microbial biofilm and its importance for food microbiology

Syed Ehtasham Amin[1],Muhammad Ismail[2],Shahzad Haider[3],Sittara Noori Naqvi[1],Haris shahzad[4],Ammad Amin[1],Bilal Ahmed[5], Rizwan Ullah[6],Abdul Haseeb[5], Naveed ul Haq[7],Muhammad Usman Shoukat[1],Arsalan Fareed[1], Arsalan Khan[1,]Malick Faizan Shoukat[4]

1.Department of food science and technology, The University of Haripur,Pakistan

2.Department of Microbiology, Sarhad University of science and information technology,Peshawar,Pakistan

3.Department of Agronomy, The University of Haripur,Pakistan

4.Department of Horticulture, The University of Haripur,Pakistan

5.Department of Medical lab technology,Abbottabad University of science and technology,Pakistan

6.Department of Microbiology,Abbottabad University of science and technology,Pakistan

7.Department of food science,The University of Guelph,Canada

Abstract

Due to the possibility of spoilage bacteria and pathogenic bacteria contaminating food items, biofilms are an important risk issue in the food business. Most bacteria may stick to surfaces and create biofilms, and live for short or even longer, depending on the kind of bacterium surrounding circumstances. Multiple developmental phases, including as primary attachment, mating, maintenance, and dispersion, are included in the life cycle of biofilms. Because bacteria of biofilms are often well shielded from stress of the environment, they are very challenging to eliminate and find in the food sector. The strategies and medications used to stop and stop the formation of biofilms are provided and addressed in the current publication. Furthermore, a number of cutting-edge methods, such as confocal laser scanning microscopy, polymerase chain reaction, DNA microarray, and, have recently been used to identify and assess bacteria adhered to surfaces. The prevention and management of food-related spoilage and pathogenic microorganisms can benefit from greater understanding of the structural, physiological, and molecular communication in biofilms. The current work emphasises fundamental and applied ideas that are crucial for comprehending how biofilms affect bacterial life, and spread in environments involved in processing of the food.

Key words: Biofilm, Food microbiology, Food spoilage, Bacteria.

Introduction

Bacteria can adapt their demands for existence in many settings with remarkable ease. The capacity of the microbe to create biofilms is one of the most significant microbial traits allowing these adaptations since it makes it easier to adapt to challenging environmental circumstances.

A lot of work has been done in recent years to better understand microbial biofilms, which are described as intricate and organized communities in biology contained in an polymeric matrix that may form on wet surfaces that are either biotic or abiotic. Because bacteria may stick to practically any surface, including wood and food items, the development of biofilms in the food business is frequent and causes worry. Bacterial foodborne illnesses are more likely to arise and cause economic losses when they are attached to food and contact surfaces. For instance, 59% of food-borne illness outbreaks evaluated in France had equipment contamination with biofilms as a significant cause. Since they are shielded from environmental stressors like UV radiation, dehydration, or treatment with antibacterial and sanitising chemicals, bacteria in biofilms provide their members a survival advantage, which makes getting rid of them extremely difficult. Bacteria in biofilms may also be useful for biotechnological applications and for the food business. For instance, it aids in the waste treatment process and the manufacture of fermented foods.

Biofilm in the Food Industry

There are significant ramifications when bacteria cling to surfaces used in the food business and subsequently form biofilms. Such organised microbial communities are an opportunity for raw materials and processed food to get contaminated goods as they go through different phases of food manufacturing activities. They serve as a reservoir of microorganisms in food processing facilities. Additionally, the existence of biofilms may result in food spoiling, financial losses, shortened product shelf lives, or disease transmission. Salmonella spability .'s to stick to surfaces was originally documented in a research on pathogenic bacterial biofilms [46]. Since then, several bacteria, including Listeria monocytogenes, shown to produce biofilm in food producing facilities. Escherichia coli, Campylobacter jejuni, Staphylococcus spp., and Yersinia enterocolitica [47, 161].

L. monocytogene is detected in environment of food company facilities and been isolated from dairy processing facilities. This microbe has strong adhesion to inert surfaces and has been

shown to persist for a long time in sessile form (32, 58, 71, 97, 129, 161, 176). For instance, Unnerstad et al. [177] discovered similar L. monocytogenes clone surviving for 7 years in a dairy facility. Additionally, Miettinen et al. [121] showed that isolates of L. monocytogenes PFGE type II have endured for at least 7 years in an ice cream factory.

Common food borne microbes in the food industry is Staphylococcus aureus [116, 152]. Researchers have shown that the Staphylococcus genus may attach to surfaces and produce biofilms [116]. In their investigation of biofilm development in S. aureus strains associated with food and clinical settings grown under various stresses such as temperature and ethanol. Rode et al. [152] found that certain food additives, including sodium chloride and glucose, may encourage S. aureus to form biofilms. Exopolysaccharides (EPS) and biofilm formation by E. coli occur on food surfaces and machinery utilised in beef companies [20, 44]. Earlier this year, Mendonça et al. [117] also showed that E. coli O157:H7 had the ability to form biofilm on various surfaces frequently used by the food industry, and Dourou et al. [44] found that E. coli indicating the necessity for more comprehensive cleanliness programmes. In addition to the places where cattle is slaughtered (15 °C), attachment also happened during cold storage (4 °C).

A problem for the food business is that lactic-acid bacteria (LAB) may result in biofilm, which might lead to unnecessary change in foods [87, 95]. One such instance is the non-starter lactic acid bacteria Lactobacillus curvatus, which may create calcium lactate crystals and the isomer D-()-lactic acid, which causes biofilm to grow and may cause sensory abnormalities in cheese [95]. Additionally, Bacillus biofilms production has been described, for example, in the powder of milk and in processing plants of whey, indicate a risk for foodborne illnesses [47, 101, 158].

It is also important to emphasise the advantages of biofilms for the food business, which are connected to biotechnological applications. For example, while making vinegar, the bacteria of acetic acid produce on the wood chips, and the attachments of bacterias promotes a more effective formation of acid from substrate. Biofilms are crucial components in the creation of fermented foods [172]. Additionally, findings from investigations by Demirci et al. [41] showed that Saccharomyces cerevisiae's synthesis of ethanol in biofilms had advantages over traditional fermentation due to its increased productivity. Since Asper gillus niger biofilms established on polyester fabric produced 70% higher cellulose activity than easily floating mycelial culture,

Gamarra et al. [53] conclude that Aspergillus niger biofilms may be exploited for industrial manufacture of celluloses. Due to uses in companies in the processing of food, textiles, washing, flesh, and paper, these enzymes are significant. According to Morikawa [125], the prevention of infection brought on by the pathogens of plant, the decrease of steel corrosion, and the use of new chemicals are positive factors connected to biofilm development by B. subtilis and other bacilli. Controlling growth of harmful biofilms is crucial, yet good biofilms produced by industrial microorganisms may aid in the creation of novel biotechnological procedures.

Biofilm Formation

The term "biofilm" refers to an organised bacterial community that is attached to sides or other objects and fixed in an exopolysaccharide medium [38]. Their development entails the following steps: (1) initial planktonic cell attachment on surface (2) creation of extracellular polimeric medium (3) establishment of microcolonies and chemical signal secretion; (4) maturation of biofilm architecture; and (5) cell dispersion [62, 68, 123, 154]. However, several elements that will be covered in greater detail will need to be taken into record in this procedure of biofilm production.

Biofilm Architecture

On both biotic and abiotic surfaces, pathogenic biofilms produced by bacteria such Salmonella spp., L. monocytogene, S. aureus, E. coli O157:H7, Bacillus cereus, and Vibrio cholerae had been well researched [10, 17, 25, 164, 168, 179]. However, several pathogens, including Chronobacter sakasakii, B. cereus, and C. jejuni, can form detached totals and pellicle at the air-liquid edge [79, 96, 187]. Bacteria often produce non-uniformly constructed biofilms that vary in thickness, cell distribution, depending on the bacterial strain, genetic characteristics, environmental factors, as well as the experimental model employed in laboratory experiments (Table 1). A heterogeneity structural picture of microbial biofilms is provided by microscopic techniques including scanning electron microscopy, confocal laser Cry embedding and scanning microscopy were followed by sectioning and microscopic examination.. In contrast to SEM, CSLM and cry embedding techniques offer a greater capacity to photograph the inside of the biofilm and a greater capability to deliver quantitative data [17, 65, 66, 171].

Mature biofilms could previously be observed using a microscope method as an intriguing architecture comprised of clusters and edges, or more complicated micro colonies grouped in stalk-like or mushroom-like forms. Additionally, cell-free holes and networks in the biofilm's core may be seen, which may indicate a location for nutrition and waste exchange as well as local cell dispersion. For the organisation and maintenance of biofilms, certain cell free regions can be discovered full with EPS [17, 68, 69, 91, 171].

Factors Involved in Biofilm Formation: Surface Properties, Environmental Factors and EPS

Because they affect the first cell attachment, the physicochemical properties of bacterium and hard sides in the food business are crucial for biofilm development [1]. A few good examples of surface characteristics include hydrophobicity, cation bridging, roughness, and topography [133, 150, 181]. However, it is acknowledged that surface materials metal, plastic etc. frequently used in home or in the industry of food processing have a part in the retention of pathogens of foodborne [160, 174]. There is no agreement on whether bacteria can attach to hydrophilic and hydrophobic surfaces. Stainless steel type 304 is the contact material used in the food business because it is corrosion-resistant, easy to clean, and chemically occur at a range of processing temperatures [194].

Table 1: Biofilm architecture of some foodborne pathogens according to experimental laboratory model used

Bacteria	Experimental Model	Biofilm architecture	Reference
C. Sakasakii	Static condition Flow condition	Pellicles and flocks. Basal Layer of cells and Micro colonies.	(96) (70)
L. monocytogenes	Static condition Flow condition	Homogeneous layer and micro colonies of rod cells. Ball-shaped micro-colonies surrounded by network of knitted chains composed of elongated cells.	(151)
C. perfringens	Static condition	Flat and thickness biofilm encased in a dense EPS.	(179)
P. aeruginosa	Static condition Flow condition	Flat biofilm during early colonization.	(106)

		Mushrooms like micro colonies surrounded by water-filled voids latter.	
S. aureus	Static and flow condition	Dense layer of cells	(144)

However, in general, because of constant usage, the material's topography exhibits fissures and crevices that shield the germs from mechinary cleaning techniques and sanitising treatments [161]. Additionally, some food processing tools and equipment, such as dicers, rollers, and conveyor belts, may contain difficult-to-clean inaccessible places [32, 165]. Additionally, any leftover food particles or particles, such as proteins in milk of beef, might be adsorbent to the surface and build a conditioning layer on food processing. Typically beginning during the first five to ten seconds of contact, this early stage may offer a niche where microorganisms can quickly proliferate [82, 88, 165, 194]. Thus, food medium can significantly hinder the sanitising and cleaning of surfaces used in food preparation. Additionally, bacteria may sense surfaces and/or connect to them by using sporulation, cellular membrane constituents such as protein and supplements (such as flagella, pili, fimbriae, and curli fibres) [15, 19, 36, 78, 138, 157, 166, 179, 187].

Additionally, various environmental parameters, such as pH, temperature, the amount of nutrients in the medium, and the population features of bacteria, have a effect on the physicochemical features of solid surfaces as well as cell wall modification [34, 64, 81, 83, 152]. Between various and different shapes of the same species, the EPS volume, arrangement, chemical makeup, and physical characteristics might change greatly [40, 49, 111, 114, 150]. Among Gram-positive bacteria, staphylococci are among the best EPS producers, creating a slime with teichoic acid, small amounts of proteins, and a concoction of polycationic exopolysaccharide and polysaccharide intercellular adhesin (PIA) known as poly-N-acetylglucosamine (PNAG), and polysaccharide intercellular adhesin (PIA) [135]. Similar to PIA, adhesin-like polymers appear to be produced by some species of Gram-negative bacteria, such as E. coli [185]. However, since it was identified by Whitchurch et al. [186] as a significant component of structure of P. aeruginosa biofilm, the eDNA has received the most attention as an element of EPS. Other pathogens, such as B. cereus, S. aureus, L. monocytogenes, and V. cholerae, have recently had their role and

potential source revealed [21, 39, 67, 106, 144, 156, 182]. Therefore, understanding these unique matrix components' roles in biofilm formation and dispersal as well as their interactions with other cell components may help researchers come up with fresh ways to stop and manage biofilms.

Bacterial Biofilm Dispersal

Bacterial dispersion might happen in the environment spontaneously when biofilms form,most likely as a result of hazardous waste buildup or nutritional deficiency [81, 155]. According to Kaplan [80], active or passive processes are often responsible for biofilm dissemination. The former is connected to bacterial structures and the synthesis of extracellular chemicals (e.g., signalling molecules). The latters is affected by outside factors via several processes 1) Sloughing, also known as the quick and extensive elimination of significant areas of the biofilm, is most frequently done in the later phases of biofilm production.; 2Shearing or erosion, which describes the regular elimination of a biofilm's one cell or tiny branches of cells at low levels throughout the biofilm growth process.; and (3) Abrasion is the term for the detachment brought on by solid particles slamming into the biofilm. When bacterial contamination is brought about by human interaction in medical devices or food processing facilities, chemical, physical, or biological cleanup can also be utilised [29, 63]. Additionally, in single-species and multi-species biofilms by creating interspecific antimicrobial chemicals, intimate connections based on competition, mutualism may cause cell dispersion matrix-degrading enzymes or quorum sensing (QS) sig nals [30, 112, 115, 124, 173, 178]. As a result, it emphasises the fact that a variety of elements are "necessary" or "needed" for the dispersal of biofilm, and some of them are crucial to successfully removing primary molded biofilms on hard exteriors.

Methods to Prevent and to Control Cells in Biofilms

For regulating food quality and safety, Good Manufacturing Practice and Hazard Analysis and Critical Control Points have been devised [158]. Identifying the Critical Points where biofilms might grow and evolve is one of the first stages in preventing and controlling them [158, 194]. Compared to their planktonic counterparts, microorganisms in biofilms are 1,000 times more resistant to disinfectants. The synthesis of enzymes that break down antimicrobial compounds, decreased diffusion, anaerobic growth, physiological changes brought on by slower growth rates, and other characteristics of the physiology of biofilms [73, 88, 163, 165]. The most popular disinfectants used by the food industry, such as quaternary ammonium compounds, ozone,

peracetic acid, phenols, and biguanidines, are insufficient to remove the biofilms as a result of those factors [129, 161, 189, 194]. As a result, a combination of sanitizers and other techniques is occasionally necessary [118, 161, 163]. Because of their capacity to pierce and reach tight spaces, steam vapour technology and aerosolized sanitizer are promising uses for disinfecting biofilms on environmental surfaces [141].

According to Park et al. [141], treatment along 100 ppm of per acetic acid was much successful than the same dosage of sodium hypochlorite in inactivating biofilm-borne L. monocytogenes, Salmonella Typhimurium, and E. coli O157:H7. A procedure known as Clean-In-Place (CIP) is used in dairy manufacturing facilities (DMP) to reduce biofilm. CIP comprises cleaning and sanitising using a mix of machine-driven, current, and chemical processes [24, 88, 95, 128]. Preventing cell adherence by altering the chemical composition of surfaces is another method for managing biofilms [27, 163]. Surfactants have a basic structural property that includes both a hydrophilic and hydrophobic arrangement, and they may aid in enhancing cleaning processes through through emulsification and wetting [27, 163]. It has been discovered that the biosurfactants generated by a wide range of bacteria, actinobacteria, and fungi may be helpful in preventing the development of biofilms [57, 161, 167].

According to Gómez et al. [58], pre conditioning polystyrene exteriors with surfactin (0.25%) decreased L. monocytogenes and S. enteritidis adherence by 42.0%, whereas treatment with rhamnolipids (1.0%) reduced L. monocytogenes adhesion by 57.8% and S. aureus union by 67.8%. Molecular and genetic underpinnings of pathogenic biofilm development are the present focus of research since they may help with the creation of fresh methods to obstruct important biofilm growth pathways [70, 90, 94, 97, 176]. Although the molecular processes by which the majority of foodborne pathogen develop biofilms are not well understood, it is identified that cellulose and aggregative fimbriae (Tafi) are two predominant matrix components of Salmonella biofilms [168, 169]. In S. aureus, the development of biofilms is regulated by a number of genes, primarily sarA, agr, ica, and sigB [2].

According to the research, QS systems may influence the establishment of biofilms as well as networks that regulate sporulation, competence, and virulence [2, 84, 161, 169]. Due to the uncertainty surrounding their safety, Other microbial, vegetal, and animal species release QS inhibitors, along with Quorum Quenching enzymes, which cause the enzymatic decimation of

indication particles, are also a very appealing way for control and regulation of biofilms, though this is still a challenge [2, 81, 93, 161]. Enzymatic cleaning has the potential to be employed in the food sector as an another technique to eliminate biofilms. Cryotin, and krilltrypsin were tested in contradiction of biofilms of Lactobacillus bulgaricus, Lactobacillus lactis, and Streptococcus thermophilus by Augustin et al. [11]. Despite the positive outcomes mentioned by the writers, the usage of enzymes is quiet restricted since it can be challenging to determine which enzymes are most efficient against certain forms of biofilms and because they are expensive. [118, 161].

Bacteriophages, viruses that specifically mark bacteria, may also be a viable approach for eliminating undesired bacteria in biofilms. The foundation of phage treatment is the use of lytic-phages that have the capacity to compromise the structural integrity of the EPS matrix seen in biofilms [43, 147, 161]. According to Pires et al. [147], lytic phages were isolated and characterised, and they were able to infect antibiotic-resistant P. aeruginosa strains and cause a 3 log drop in cell counts in biofilms. Due to their ability to regulate biofilm, antimicrobial compounds known as bacteriocins have received much research [55, 161]. Although the precise mode of action in biofilms is still not fully understood, bacteriocins are well recognized to create holes in the bacterial cell membrane [3]. Winkelströter et al. [188] showed that L. monocytogenes biofilm development may be inhibited by culture supernatant covering bacteriocin generated by Lactobacillus sakei 1. Spray-dried Lactococcus lactis UQ2 or Lactococcus lactis UQ2 decreased L. monocytogenes Scott A planktonic and sessile cells adhered to stainless steel chips by more than 5 log [55]. To control bacteria in biofilms, several natural antimicrobial substances have been investigated [8, 20]. After 24 hours of exposure, Laird et al. [89] showed that the number of Enterococcussp. and S. aureus in biofilms were decreased by 1.5 and 3 log by the essential oil vapours of orange/bergamot (1:1, v/v). Sugar fatty acid esters decreased S. aureus adhesion, as demonstrated by Furukawa et al. in their study (51). Monocytogenes, L. and Streptococcus mutans on an abiotic surface. Scallop shell powder (SSP) was shown by Bodur and CagriMehmetoglu [20] to reduce the growth of L. monocytogenes, S. aureus, and E. coli O157:H7 in biofilms on stainless steel surfaces, while acidic and neutral electrolyzed waters were shown by Arevalos-Sánchez et al. [8] to has significant antibacterial action against listerial biofilms at doses of 65 ppm or higher. Although progress has been made to understand the processes that govern undesired biofilms, additional research is still required in this field because no strategy is thought to be 100% successful. Techniques for Finding and counting bacterium in Biofilms. Sessile cells be removed

from exteriors using techniques including sonication, sponge swabbing, and agar plating or enrichment for pathogen identification. The swabbing approach may not be effective in removing all bacteria, according to some authors [26, 50, 56, 119, 137], and using an ultrasonic device may produce more consistent results [9, 136, 137]. The combined swab-vortex method outperformed shaking, vortexing with glass beads, vortex and sonication in removing germs from stainless steel, according to Luppens et al. [104].Other writers [100, 122] did not discover any appreciable variations between these approaches. By using direct microscopic inspection, Bremer et al. [24] confirmed that after the vortexing procedure but not after the swabbing approach, mixed biofilms of bacteria persisted on the surface under study. By using a SEM, Lindsay and von Holy [100] found shaked beads unconcerned cells and EPS remains from the exteriors more effectively than vortexing.

Numerous microscopy methods, including fluorescent microscopy, CLSM, and scanning electron microscopy, have been used to qualitatively and/or quantitatively analyse the architecture of biofilms on various surfaces. Examining fluorescent specimens requires the use of CLSM and fluorescence microscopy. Fluorescence microscopy can only analyse multilayered biofilms in two dimensions, but CLSM enables quantitative visualisation of biofilm reconstructions in two-dimensional, three-dimensional and four-dimensional space without causing any invasiveness to the subject [45, 93]. Calculations of the biofilm's volume, volume to surface ratio, roughness ratio , mean thickness, and maximum thickness are also possible, but they rely on the availability of specialised software and the user's knowledge of how to use it [18, 92]. The most widely used fluorescent probes for determining the total number of bacteria in biofilms are those that mark DNA and RNA with acridine orange (AO) or other DNA-specific dyes The most popular feasibility-staining methods for biofilm tasters are CTC-DAPI (5-cyano-2,3-ditolyl tetrazolium chloride and 4′,6-diamidino-2-phenyindole and the LIVE/DEAD bacterial viability kit Bac LightTM, is used to distinguish between total cells and living cells by noticing metabolic activity and cell viability, respectively. Binding of total cells (SYTO 9, SYTO 63), dead cells (SYTO X blue), proteins (SYPRO Ruby and fluorescein isothiocyanate [FITC]), lipids (DiD, Nile red), and extracellular-polysaccharides (EPS) (Calcofluor white, concanavalin A-tetramethylrhodamine conjugate), a variety of fluorophores are also used to characterize biofilm. Genetically modifying microorganisms to show fluorescent protein (FP), which didn't need substrate or other factors for its activitation, is another option for studying microorganisms in biofilms [162]. Multi-species

biofilms are also studied utilising dual approaches that employ two FP variations or one FP different paired with a glowing dye. [38, 61, 86, 105, 108, 126, 151].

Light microscopy struggles to capture the intricate ultrastructure of biofilms, while electron microscopy, such as SEM, has a greater picture and can reveal position of a solo bacterial cell as well as the three-dimensional (3D) structure of the biofilm. However, the biofilm samples must be dehydrated during preparation for SEM examinations, and the biofilm structure may change [5]. There isn't a single technique that enables you to see the structure of the biofilm matrix, according to Alhede et al [5] .'s comparison of four multiple SEM techniques: conventional SEM, Focused Ion Beam (FIB)-SEM, CLSM with SEM techniques [cryo-SEM and environmentalSEM (ESEM), and SEM with CLSM. Lawrence [91] further showed that multimicroscopic study is required to fully understand the structure and content of biofilms. AFM is a different high-resolution imaging method that uses a straightforward sample preparation process [190] and is effective for assessing important parameters such size of cell, and surface roughness [46]. The incapacity to acquire a broad area review scan, the inability to examine the dividers of bacterial cells, and the easy character of the biofilm are some of the limitations of AFM [191]. Dáz et al. [42] used an AFM to study the flagella's direction in the biofilm that P. fluorescens developed on hard, dense exteriors without taster pre-cure. Teixeira et al. [174] employed AFM to evaluate the topography and roughness of surfaces quantitatively, but they struggled to connect the dots between surface characteristics and adhesion strength. AFM may provide good resolution pictures of P. aeruginosa type IV pili and is adequate to explore their elastic characteristics, according to research by Touhami et al. [175]. By hybridising to ribosomal RNA, FISH is now a common molecular technique for locating and measuring specific genera in microbial populations. In Phylogenetic markers at the 16S and 23S rDNA sequences are used to create rRNA-targeted oligonucleotide probes for FISH, or peptide nucleic acid fluorescence in situ hybridization. (PNA-FISH) [59, 107, 139, 153]. These probes are labelled with a fluorescent dye (Cy3, FAM, FITC, rhodamine), an enzyme, or both. Shorter peptides are used in the PNA-FISH approach, which has greater specificity and understanding than traditional DNA probes [6, 110]. In order to prevent food contamination, methods for quick foodborne pathogen detection on surfaces are crucial. Molecular techniques, such as real-time polymerase chain reactions, can be used as an alternative to quickly identify and measure the presence of bacterial pathogens on contaminated surfaces (RT-PCR). Reactions are carried out in a 1 tube system, this approach is quick, sensitive, and specific, and it

minimises post-PCR contamination. It can also identify a small number of germs on surfaces. DNA from dying cells is amplified leads to false-positive PCR findings, a different approach to prevent Apply an improvement method that rises concentrations of targeted cells and only lets for the detection of living bacteria [60, 109]. Additionally, based on membrane integrity, PCR has been utilised to quantify living cells in biofilms as descripted by Nogva et al. [131]. These researchers employed the DNA intercalating dyes ethidium monoazide (EMA) and propidium monoazide (PMA), which only enter cells with damaged membranes (dead cells). Quantitative PCR (qPCR) is used to extract genomic DNA and analyse it without include cells with damaged membranes in the study. Because some bacterial species' living cells may also be penetrated by EMA, which causes significant DNA loss, the PMA assay may have a significant benefit done the EMA assess [130, 140].

Using qRT-PCR and fluorescent dyes, several papers have estimated the number of duplicates of a mark gene and evaluated the quantity of RNA transcripts of specific genes to calculate the bacterial numbers in biofilms. Readings on gene appearance in biofilms may target specific genes using different fluorescently labelled probes, and the usage of double-categorized probes lets the simultaneous examination of several genes. [60, 98, 143]. Gene expression data from microarrays may be verified using qRT-PCR, which has a wide dynamic range. Another molecular technique that makes it possible to concurrently analyse several genes is the DNA microarray. Microarrays, which are composed of hundreds of different DNA arrangements, each one linked at a identified place to a tiny solid surface, may be used to learn about the genetic foundation of microbial diversity, evolution, and epidemiology. An indication of the nucleic acid sequences can be produced by complementary, tagged mRNA or DNA binding to the static arrangements, creating a gene expression profile for a particular microbe. [13, 103, 104, 191].

Compared to biofilm cells and planktonic cells, or mutant vs. wild-type cells, microarrays have mostly been employed to analyse changes in gene expression caused by environmental stressors or treatments that imitate circumstances seen in the food business [149, 184, 192, 193]. In more recent years, a large number of writers have employed molecular techniques and proteomic tests to clarify several biofilm structural components, regulatory mechanisms, and signalling molecules involved in the development of biofilms [72, 76, 183, 192]. In order to grow and quantify biofilms using 96-well microtiter plates, a number of high-throughput techniques

have been developed. These techniques include (aSYTO 9 [22], the BioFilm Ring Test®, and the Biofilm Biomass Assay, which assesses living and dead cells as well as medium stained with crystal violet (CV) [99, 170]. Resazurin or Alamar Blue [132], the XTT test [4, 52, 146], and non-fluorescent fluorescein diacetate (FDA) [74, 142] are examples of such tests. are all methods for quantifying live cells.

The 1,9-dimethyl methylene blue (DMMB) dye, which is produced when a decomplexation solution is added and indicates the quantity of polysaccharides that occur in the biofilm matrix, may be used to quantify the biofilm matrix [14]. Broad application and high repeatability in microtiter plate tests for resazurin, XTT, FDA, SYTO 9, and DMMB were confirmed by Peeters et al. [142]. Due to fewer handling procedures, including no washing or staining, the BioFilm Ring Test® was quicker than the CV technique [33]. The Alamar Blue technique has a number of advantages over other approaches, absence of chemical toxicity, and viability assessment [146]. Burton et al. [28] established a spectro fluorometric method to measure bacteria growing in microtiter plates and discovered that it was further complex and specific than CV stain. They stained bacterial biofilms with wheat germ agglutinin-Alexa Fluor 488 conjugate, which specifically binds to N-acetylglucosamine remains in biofilms. To see and measure biofilms, some authors combined specialized microplates with microscopy, such as the Calgary Biofilm Device (CBD) and SEM [31] or CLSM and SEM [68]. Benoit et al. [16] created a Bioflux system with regulated flow rates and real-time viability determination that permits microscopic analysis, whereas Bridier et al. [25] employed a technique paired with CLSM. examination.

Conclusion

In the surroundings of the food business, several bacteria are able to stick and form biofilms, which may serve as a significant reservoir for adulteration of food items. Biofilms exhibit a tremendous degree of difficulty, and many of the underlying processes are still poorly understood. Recent years have seen a significant increase in the use of genomic and proteomic analysis to screen and discover genes associated with biofilm development or dispersion. In an effort to manage the microorganisms in biofilms, these novel approaches may improve our study of the molecular underpinnings of the regulatory pathways in biofilms. They will also accelerate the creation of new tactics and technology.

References

1. Abban S, Jakobsen M, Jespersen L (2012) Attachment behavior of Echerichia coli K12 and Salmonella Typhimurium P6 on food contact surfaces for food transportation. Food Microbiol 31:139– 147

2. Abee T, Kovács ÁT, Kuipers OP, Van Der Veen S (2011) Biofilm formation and dispersal in Gram-positive bacteria. Curr Opin Biotechnol 22:172–179

3. Abee T, Krockel L, Hill C (1995) Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. Int J Food Microbiol 2:169–185

4. Adam B, Baillie GS, Douglas LJ (2002) Mixed species biofilms of Candida albicans and Staphylococcus epidermidis. J Med Microbiol 51:344–349

5. Alhede M, Qvortrup K, Liebrechts R, Hoiby N, Givskov M, Bjarnsholt T (2012) Combination of microscopic techniques reveals a comprehensive visual impression of biofilm structure and composition. FEMS Immunol Med Microbiol 65:335–342

6. Almeida C, Azevedo NF, Santos S, Keevil CW, Vieira MJ (2011) Discriminating multi-species populations in biofilms with peptide nucleic acid fluorescence in situ hybridization (PNA FISH). PLoS ONE 6:14786

7. Anderson JM, Lin Y, Gillman AN, Parks PJ, Schlievert PM, Peterson ML (2012) Alpha-toxin promotes Staphylococcus aureus mucosal biofilm formation. Front Cell Infect Microbiol 2:64–69

8. Arevalos-Sánchez M, Regalado C, Martin SE, DomínguezDomínguez J, García-Almendárez BE (2012) Effect of neutral

electrolyzed water and nisin on Listeria monocytogenes biofilms, and on listeriolysin O activity. Food Control 24:116–122

9. Asséré A, Oulahal N, Carpentier B (2008) Comparative evaluation of methods for counting surviving biofilm cells adhering to a polyvinyl chloride surface exposed to chlorine or drying. J Appl Microbiol 104:1692–1702

10. Auger S, Ramarao N, Faille C, Fouet A, Aymerich S, Gohar M (2009) Biofilm formation and cell surface properties among pathogenic and nonpathogenic strains of the Bacillus cereus group. Appl Environ Microbiol 75:6616–6618

11. Augustin M, Ali-Vehmas T, Atroshi F (2004) Assessment of enzymatic cleaning agents and disinfectants against bacterial biofilms. J Pharm Sci 18:55–64

12. Baird FJ, Wadsworth MP, Hill JE (2012) Evaluation and optimization of multiple fluorophore analysis of a Pseudomonas aeruginosa biofilm. J Microbiol Methods 90:192–196

13. Ball KD, Trevors JT (2002) Bacterial genomics: the use of DNA microarrays and bacterial artificial chromosomes. J Microbiol Methods 49:275–284

14. Barbosa I, Garcia S, Barbier-Chassefière V, Caruelle JP, Martelly I, Papy-García D (2003) Improved and simple micro assay for sulfated glycosaminoglycans quantification in biological extracts and its use in skin and muscle tissue studies. Glycobiology 13:647–653

15. Barken KB, Pamp SJ, Yang L, Gjermansen M, Bertrand JJ, Klausen M, Givskov M, Whitchurch CB, Engel JN, Tolker-Nielsen T (2008) Roles of type IV pili, flagellum-mediated motility and extracellular DNA in the formation of mature multicellular structures in Pseudomonas aeruginosa biofilms. Environ Microbiol 10:2331–2343

16. Benoit MR, Conant CG, Ionescu-Zanetti C, Schwartz M, Matin A (2010) New device for high-throughput viability screening of flow biofilms. Appl Environ Microbiol 76:4136–4142

17. Berk V, Fong JCN, Dempsey GT, Develioglu ON, Zhuang X, Liphardt J, Yildiz FH, Chu S (2012) Molecular architecture and

assembly principles of Vibrio cholera biofilms. Science 337:236–239

18. Beyenal H, Donovan C, Lewandowski Z, Harkin G (2004) Threedimensional biofilm structure quantification. Journal of

Microbiological Method 59:395–413 Microbial Biofilms of Importance 41

19. Biswas R, Agarwa RK, Bhilegaonkar KN, Kumar A, Nambiar P, Rawat S, Singh M (2010) Cloning and sequencing of biofilmassociated protein (bapA) gene and its occurrence in different serotypes of Salmonella. Lett Appl Microbiol 52:138–143

20. Bodur T, Cagri-Mehmetoglu A (2012) Removal of Listeria monocytogenes, Staphylococcus aureus and Escherichia coli

O157:H7 biofilms on stainless steel using scallop shell powder. Food Control 25:1–9

21. Boles BR, Thoendel M, Roth AJ, Horswill AR (2010) Identification of genes involved in polysaccharide-independent Staphylococcus aureus biofilm formation. PLoS ONE 5:e10146

22. Boulos L, Prévost M, Barbeau B, Coallier J, Desjardins R (1999) LIVE/DEAD® BacLightTM: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. J Microbiol Methods 37:77–86

23. Bredholt S (1999) Microbial methods for assessment of cleaning and disinfection of food-processing surfaces cleaned in a lowpressure system. Eur Food Res Technol 209:145–152

24. Bremer PJ, Monk I, Osborne CM (2001) Survival of Listeria monocytogenes attached to stainless steel surfaces in the presence or absence of Flavobacterium spp. J Food Prot 64:1369–1376

25. Bridier A, Dubois-Brissonnet F, Boubetra A, Thomas V, Briandet R (2010) The biofilm architecture of sixty opportunistic pathogens deciphered using a high throughput CLSM method. J Microbiol Methods 82:64–70

26. Brooks JD, Flint SH (2008) Biofilms in the food industry: problems and potential solutions. Int J Food Sci Technol 43:2163–2176

27. Bryers JD (1993) Bacterial biofilms. Curr Opin Biotechnol 4:197– 204

28. Burton E, Yakandawla N, LoVetri K, Madhyastha MS (2007) A microplate spectrofluorometric assay for bacterial biofilms. J Ind Microbiol Biotechnol 34:1–4

29. Carpentier B, Cerf P (2011) Persistence of Listeria monocytogenes in food industry equipment and premises. Int J Food Microbiol 145: 1–8

30. Carpentier B, Chassaing D (2004) Interactions in biofilms between Listeria monocytogenes and resident microorganisms from food industry premises. Int J Food Microbiol 97:111–122

31. Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A (1999) The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin

Microbiol 37:1771–1776

32. Chaturongkasumrit Y, Takahashi H, Keeratipibul S, Kuda T, Kimura B (2011) The effect of polyesterurethane belt surface

roughness on Listeria monocytogenes biofilm formation and its cleaning efficiency. Food Control 22:1893–1899

33. Chavant P, Gaillard-Martinie B, Talon R, Hébraud M, Bernardi T (2007) A new device for rapid evaluation of biofilm formation potential by bacteria. J Microbiol Methods 68:605–612

34. Chavant P, Martinie B, Meylheuc T, Bellon-Fontaine MN, Hebraud M (2002) L. monocytogenes LO28: surface physicochemical properties and ability to form biofilms at different temperatures and growth phases. Applied Environmental Microbiology 68:728–737

35. Chen MY, Lee DJ, Tay JH, Show KY (2007) Staining of extracellular polymeric substances and cells in bioaggregates.Appl Microbiol Biotechnol 75:467–474

36. Cookson AL, Cooley WA, Woodward MJ (2002) The role of type 1 and curli fimbriae of Shiga toxin-producing Escherichia coli in adherence to abiotic surfaces. International Journal of Medical Microbiology 292:195–205

37. Costerton JW, Lewandowski Z, Caldwell D, Korber DR, Lappinscott HM (1995) Microbial biofilms. Annual Review

Microbiology 49:711–745

38. Cowan SE, Gilbert E, Khlebnikov A, Keasling JD (2000) Dual labeling with green fluorescent proteins for confocal microscopy. Appl Environ Microbiol 66:413–418

39. Das T, Sharma PK, Busscher HJ, van der Mei HC, Krom BP (2010) Role of extracellular DNA in initial bacterial adhesion and surface aggregation. Appl Environ Microbiol 76:3405–3408

40. Decker E-M, Dietrich I, Klein C, von Ohle C (2011) Dynamic production of soluble extracellular polysaccharides by Streptococcus mutans. International Journal of Dentistry 46:16

41. Demirci A, Pometto AL, Ho KL (1997) Ethanol production by Saccharomyces cerevisiae in biofilm reactors. J Ind Microbiol Biotechnol 4:299–304

42. Díaz C, Schilardi PL, Salvarezza RC, Lorenzo F, de Mele M (2011) Have flagella a preferred orientation during early stages of biofilm formation?: AFM study using patterned substrates. Colloids Surf B: Biointerfaces 82:536–542

43. Donlan RM (2009) Preventing biofilms of clinically relevant organisms using bacteriophage. Trends Microbiol 17:66–72

44. Dourou D, Beauchamp CS, Yoon Y, Geornaras I, Belk KE, Smith GC, Nychas G-JE, Sofos JN (2011) Attachment and biofilm formation by Escherichia coli O157:H7 at different temperatures, on various food-contact surfaces encountered in beef processing. Int J Food Microbiol 149:262–268

45. Dufrêne YF (2003) Recent progress in the application of atomic force microscopy imaging and force spectroscopy to microbiology. Curr Opin Microbiol 6:317–323

46. Duguid PJ, Anderson ES, Campbell I (1966) Fimbriae and adhesive properties in salmonellae. The Journal of Pathology and Bacteriology 92:107–137

47. Elhariry HM (2011) Attachment strength and biofilm forming ability of Bacillus cereus on green-leafy vegetables: cabbage and lettuce. Food Microbiol 28:1266–1274

48. Feng L, Wu Z, Yu X (2013) Quorum sensing in water and wastewater treatment biofilms. J Environ Biol 34:437–444

49. Flemming HC, Wingender J (2010) The biofilm matrix. Nature Reviews 8:623–633

50. Flint S (2006) A rapid, two-hour method for the enumeration of total viable bacteria in samples from commercial milk powder and whey protein concentrate powder manufacturing plants. Int Dairy J 16: 379–384

51. Furukawa S, Akiyoshi Y, O'Toole GA, Ogihara H, Morinaga Y (2010) Sugar fatty acid esters inhibit biofilm formation by foodborne pathogenic bacteria. Int J Food Microbiol 138:176–180

52. Gabrielson J, Hart M, Jarelöv A, Kühn I, McKenzie D, Möllby R (2002) Evaluation of redox indicators and the use of digital scanners and spectrophotometer for quantification of microbial growth in microplates. J Microbiol Methods 50:63–73

53. Gamarra NN, Villena GK, Gutiérrez-Correa M (2010) Cellulase production by Aspergillus niger in biofilm, solid-state, and submerged fermentations. Appl Microbiol Biotechnol 87:545–551

54. Gandhi M, Chikindas ML (2007) Listeria: a foodborne pathogen that knows how to survive. Int J Food Microbiol 113:1–15

55. García-Almendárez BE, Cann IKO, Martin SE, Guerrero-Legarreta I, Regalado C (2008) Effect of Lactococcus lactis UQ2 and its bacteriocin on Listeria monocytogenes biofilms. Food Control 19: 670–680

56. Garcias KS, McKillip J (2004) A review of conventional detection and enumeration methods for pathogenic bacteria in food. Can J Microbiol 50:883–890

57. Gomes MZV, Nitschke M (2012) Evaluation of rhamnolipid and surfactin to reduce the adhesion and remove biofilms of individual and mixed cultures of food pathogenic bacteria. Food Control 25: 441–447

58. Gómez D, Ariño A, Carramiñana JJ, Rota C, Yangüela J (2012) Sponge versus mini-roller for the surface microbiological control f Listeria monocytogenes, total aerobic mesophiles and Enterobacteriaceae in the meat industry. Food Control 27:242–247

59. Griffiths MW (1993) Applications of bioluminescence in the dairy industry. J Dairy Sci 76:3118–3125 42 L.K. Winkelströter et al.60. Guilbaud M, Coppet P, Bourion F, Rachman C, Prévost H, Dousset X (2005) Quantitative detection of Listeria monocytogenes in biofilms by Real-Time PCR. Appl Environ Microbiol 71:2190– 2194

61. Habimana O, Moretro T, Langsrud S, Vestby LK, Nesse LL, Heir E (2010) Micro ecosystems from feed industry surfaces: a survival and biofilm study of Salmonella versus host resident flora stains. BMC Vet Res 6:1–10

62. Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases.

Nature Review in Microbiology 2:95–108

63. Hall-Stoodley L, Stoodley P (2005) Biofilm formation and dispersal and the transmission of human pathogens. TRENDS in Microbiology 13:7–10

64. Hancock V, Witsø IL, Klemm P (2011) Biofilm formation as a function of adhesin, growth medium, substratum and strain type. International Journal of Medical Microbiology 301:570–576

65. Hannig C, Follo M, Hellwig E, Al-Ahmad A (2010) Visualization of adherent micro-organisms using different techniques. J Med Microbiol 59:1–7

66. Harmsen M, Lappann M, Knøchel S, Molin S (2010) Role of extracellular DNA during biofilm formation by Listeria

monocytogenes. Appl Environ Microbiol 76:2271–2279

67. Harmsen M, Yang L, Pamp SJ, Tolker-Nielsen T (2010) An update on Pseudomonas aeruginosa biofilm formation, tolerance, and dispersal. EMS Immunol Med Microbiol 59:253–268

68. Harrison JJ (2006) The use of microscopy and three-dimensional visualization to evaluate the structure of microbial biofilms cultivated in the Calgary Biofilm Device. Biological Procedures Online 8: 194–215

69. Harrison JJ, Turner RJ, Marques LLR, Ceri H (2005) Biofilms. Am Sci 93:508–515

70. Hartmann I, Carranza P, Lehner A, Stephan R, Eberl L, Riedel K (2010) Genes involved in Cronobacter sakazakii biofilm formation. Appl Environ Microbiol 76:2251–2261

71. Harvey J, Keenan KP, Gilmour A (2007) Assessing biofilm formation by Listeria monocytogenesstrains. Food Microbiol 24:380–392

72. Hefford MA (2005) Proteomic and microscopic analysis of biofilms formed by Listeria monocytogenes 568. Can J Microbiol 51:197– 208

73. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O (2010) Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 35:322–332

74. Honraet K, Goetghebeur E, Nelis HJ (2005) Comparison of three assays for the quantification of Candida biomass in

suspension and CDC reactor grown biofilms. J Microbiol Methods 63:287–295

75. Hou S, Liu Z, Young AW, Mark SL, Kallenbach NR, Ren D (2010) Effects of Trp- and Arg-containing antimicrobial-peptide structure on inhibition of Escherichia coli planktonic growth and biofilm formation. Appl Environ Microbiol 76:1967–1974

76. Huang Y, Shi C, Yu S, Li K, Shi X (2012) A putative MerR family regulator involved in biofilm formation in Listeria monocytogenes 4b G. Foodborne Pathogens and Disease 9:767–772

77. Jones SE, Versalovic J (2009) Probiotic Lactobacillus reuteri biofilms produce antimicrobial and anti-inflammatory factors. BMC Microbiol 9:1–9

78. Jordan SJ, Perni S, Glenn S, Fernandes I, Barbosa M, Sol M, Tenreiro RP, Chambel L, Barata B, Zilhao B, Aldsworth TG,

Adrião A, Faleiro ML, Shama G, Andrew PW (2008) Listeria monocytogenes biofilm-associated protein (BapL) may contribute to surface attachment of L. monocytogenes but is absent from many field isolates. Appl Environ Microbiol 74:5451–5456

79. Joshua GWP, Guthrie-Irons C, Karlyshev AV, Wren BW (2006) Biofilm formation in Campylobacter jejuni. Microbiology 152:387– 396

80. Kaplan JB (2010) Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses. J Dent Res 89:205–218

81. Karatan E, Watnick P (2009) Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev 73:310–347

82. Kim H, Ryu J-H, Beuchat LR (2006) Attachment of and biofilm formation by Enterobacter sakazakii on stainless steel and enteral feeding tubes. Appl Environ Microbiol 72:5846–5856

83. Kim S-H, Wei C-I (2007) Biofilm formation by multidrug-resistant Salmonella enterica serotype Typhimurium phage type DT104 and other pathogens. J Food Prot 70:22–29

84. Kim Y, Lee JW, Kang S-G, Oh S, Griffiths MW (2012) Bifidobacterium spp. influences the production of autoinducer-2

and biofilm formation by Escherichia coli O157:H7. Anaerobe 18: 539–545

85. Kirov SM (2007) Biofilm differentiation and dispersal in mucoid Pseudomonas aeruginosa isolates from patients with cystic fibrosis. Microbiology 153:3264–3274

86. Klausen M (2003) Biofilm formation by Pseudomonas aeruginosa wild type, flagella and type IV pili mutants. Mol Microbiol 48: 1511–1524

87. Kubota H, Senda S, Tokuda H, Uchiyama H, Nomura N (2009) Stress resistance of biofilm and planktonic Lactobacillus plantarum subsp. plantarum JCM 1149. Food Microbiol 26:592–597

88. Kumar CG, Anand SK (1998) Significance of microbial biofilms in food industry: a review. Int J Food Microbiol 42:9–27

89. Laird K, Armitage D, Phillips C (2012) Reduction of surface contamination and biofilms of Enterococcus sp. and

Staphylococcus aureus using a citrus-based vapour. J Hosp Infect 80:61–66

90. Lauderdale KJ, Boles BR, Cheung AL, Horswill AR (2009) Interconnections between sigma β, agr, and proteolytic activity in Staphylococcus aureus biofilm maturation. Infect Immun 77:1623– 1635

91. Lawrence JR (2003) Scanning transmission X-ray, laser scanning, and transmission electron microscopy mapping of the exopolymeric matrix of microbial biofilms. Appl Environ Microbiol 69:5543– 5554

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

93. Lazar V (2011) Quorum sensing in biofilms – how to destroy the bacterial citadels or their cohesion/power? Anaerobe 17:280–285

94. Lebeer S, Verhoeven TLA, Vélez MP, Vanderleyden J, De Keersmaecker SC J (2007) Impact of environmental and genetic

factors on biofilm formation by the probiotic strain Lactobacillus rhamnosus GG. Appl Environ Microbiol 73:6768–6775

95. Lee Wong AC (1998) Biofilms in food processing environments. J Dairy Sci 81:2765–2770

96. Lehner A, Riedel K, Eberl L, Breeuwer P, Diep B, Stephan R (2005) Biofilm formation, extracellular polysaccharide production, and cell-to-cell signaling in various Enterobacter sakazakii strains: aspects promoting environmental persistence. J Food Prot 68:2287– 2294

97. Lemon KP, Higgins DE, Kolter R (2007) Flagellar motility is critical for Listeria monocytogenes biofilm formation. J Bacteriol 189:4418–4424

98. Lenz AP, Williamson KS, Pitts B, Stewart PS, Franklin MJ (2008) Localized gene expression in Pseudomonas aeruginosa biofilms. Appl Environ Microbiol 74:4463–4471

99. Li X, Yan Z, Xu J (2003) Quantitative variation of biofilms among strains in natural populations of Candida albicans. Microbiology 149:353–362

100. Lindsay D, von Holy A (1997) Evaluation of dislodging methods for laboratory-grown bacterial biofilms. Food Microbiol 14:383– 390 Microbial Biofilms of Importance 43101. Lindsay D, von Holy A (2006) Bacterial biofilms within the clinical

setting: what healthcare professionals should know. J Hosp Infect 64:313–325

102. Lourenço A, Rego F, Brito L, Frank J (2012) Evaluation of methods to assesss the biofilm-forming ability of Listeria monocytogenes. J Food Prot 75980:1411–1417

103. Lucchini S, Thompson A, Hinton JCD (2001) Microarrays for microbiologists. Microbiology 147:1403–1414

104. Luppens SBI, Reij MW, van der Heijden RWL, Rombouts FM, Abee T (2002) Development of a standard test to assess the resistance of Staphylococcus aureus biofilm cells to disinfectants. Appl Environ Microbiol 68:4194–4200

105. Ma H, Bryers JD (2010) Non-invasive method to quantify local bacterial concentrations in a mixed culture biofilm. J Ind Microbiol Biotechnol 37:1081–1089

106. Ma L, Conover M, Lu H, Parsek MR, Bayles K, Wozniak DJ (2009) Assembly and development of the Pseudomonas aeruginosa biofilm matrix. PLoS Pathog 5:e1000354

107. MacDonald R, Brözel VS (2000) Community analysis of bacterial biofilms in a simulated recirculating cooling-water system by fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes. Water Res 34:2439–2446

108. Maeyama R, Mizunoe Y, Anderson JM, Tanaka M, Matsuda T (2004) Confocal imaging of biofilm formation process

using fluoroprobed Escherichia coli and fluoro-stained exopolysaccharide. Journal of Biomedical Materials

Research Part A 70A:274–282

109. Mafu AA, Pitre M, Sirois S (2009) Real-Time PCR as a tool for detection of pathogenic bacteria on contaminated food contact surfaces by using a single enrichment medium. J Food Prot 72: 1310–1314

110. Malic S, Hill KE, Hayes A, Percival SL, Thomas DW, Williams DW (2009) Detection and identification of specific bacteria in wound biofilms using peptide nucleic acid fluorescent in situ hybridization (PNA FISH). Microbiology 155:2603–2611

111. Mann EE, Wozniak DJ (2012) Pseudomonas biofilm matrix composition and niche biology. FEMS Microbiol Rev 36:893–916 112. Mariani C, Oulahal N, Chamba JF, Dubois-Brissonnet F, Notz E, Briandet R (2011) Inhibition of Listeria monocytogenes by resident biofilms present on wooden shelves used for cheese ripening. Food Control 22:1357–1362

113. Marsh EJ, Luo H, Wang H (2003) A three-tiered approach to differentiate Listeria monocytogenes biofilm-forming abilities. FEMS Microbiol Lett 228:203–210

114. May T, Okabe S (2008) Escherichia coli harboring a natural IncF conjugative F plasmid develops complex mature biofilms by stimulating synthesis of colonic acid and curli. J Bacteriol 190:7479–

7490

115. McDougald D, Rice SA, Barraud N, Steinberg PD, Kjellberg S (2012) Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. Nature Reviews 10:39–50

116. Meira QGS, de Medeiros BI, Alves Aguiar Athayde AJ, de Siqueira-Júnior JP, De Souza EL (2012) Influence of temperature and surface kind on biofilm formation by Staphylococcus aureus from food-contact surfaces and sensitivity to sanitizers. Food Control 25:469–475

117. Mendonça RCS, Morelli AMF, Pereira JAM, de Carvalho MM, de Souza NL (2012) Prediction of Escherichia coli O157:H7 adhesion and potential to form biofilm under experimental conditions. Food Control 23:389–396

118. Meyer B (2003) Approaches to prevention, removal and killing of biofilms. International Biodeterioration & Biodegradation 51:249– 253

119. Midelet G, Carpentier B (2002) Transfer of microorganisms, including Listeria monocytogenes, from various materials to beef. Appl Environ Microbiol 68:4015–4024

120. Midelet G, Carpentier B (2004) Impact of cleaning and disinfection agents on biofilm structure and on microbial transfer to a solid model food. J Appl Microbiol 97:262–270

121. Miettinen MK, Björkroth KJ, Korkeala HJ (1999) Characterization of Listeria monocytogenes from an ice cream plant by serotyping and pulsed-field gel electrophoresis. Int J Food Microbiol 46:187– 192

122. Moltz AG, Martin SE (2005) Formation of biofilms by Listeria monocytogenes under various growth conditions. J Food Prot 68: 92–97

123. Monds RD, O'Toole GA (2009) The developmental model of microbial biofilms: ten years of a paradigm up for review. Trends Microbiol 17:73–87

124. Moons P, Michiels CW, Aertsen A (2009) Bacterial interactions in biofilms. Crit Rev Microbiol 35:157–168

125. Morikawa M (2006) Beneficial biofilm formation by industrial bacteria Bacillus subtilis and related species. J Biosci Bioeng 1:1–8

126. Nancharaiah YV, Venugopalan VP, Wuertz S, Wilderer PA, Hausner M (2005) Compatibility of the green fluorescent protein and a general nucleic acid stain for quantitative description of a Pseudomonas putida biofilm. J Microbiol Methods 60:179–187

127. Neu TR, Swerhone GDW, Lawrence JR (2001) Assessment of lectin-binding analysis for in situ detection of glycoconjugates in biofilm systems. Microbiology 147:299–313

128. Nguyen HDN, Yuk H-G (2013) Changes in resistance of Salmonella Typhimurium biofilms formed under various conditions to industrial sanitizers. Food Control 29:236–240

129. Nilsson RE, Ross T, Bowman JP (2011) Variability in biofilm production by Listeria monocytogenes correlated to strain origin and growth conditions. Int J Food Microbiol 150:14–24

130. Nocker A, Cheung CY, Camper AK (2006) Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. J Microbiol Methods 67:310–320

131. Nogva HK, Dromtorp SM, Nissen H, Rudi K (2003) Ethidium monoazide for DNA-based differentiation of viable and dead bacteria by 5′-nuclease PCR. BioTechniques 34:804–813

132. O'Brien J, Wilson I, Orton T, Pognan F (2000) Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur J Biochem 267:5421–5426

133. Oliveira R, Azeredo J, Teixeira P.: The importance of physicochemical properties in biofilm formation and activity. In: Wuertz, S., Bishop, P. L., Wilderer, P. A. Biofilms in wastewater treatment: an interdisciplinary approach 211–231. London: IWA Publishing, (2003)

134. Orgaz B, Lobete MM, Puga CH, San Jose C (2011) Effectiveness of chitosan against mature biofilms formed by food related bacteria. Int J Mol Sci 12:817–828

135. Otto M (2008) Staphylococcal biofilms. Curr Top Microbiol Immunol 322:207–228

136. Oulahal-Lagsir N, Martial-Gros A, Boistier E, Blum LJ, Bonneau M (2000) The development of an ultrasonic apparatus for the noninvasive and repeatable removal of fouling in food processing equipment. Lett Appl Microbiol 30:47–52

137. Oulahal-Lagsir N, Martial-Gros A, Bonneau M, Blum LJ (2000) Ultrasonic methodology coupled to ATP bioluminescence for the non-invasive detection of fouling in food processing equipment – validation and application to a dairy factory. J Appl Microbiol 89: 433–441

138. Pagedar A, Singh J (2012) Influence of physiological cell stages on biofilm formation by Bacillus cereus of dairy origin. Int Dairy J 23: 30–35

139. Pamp SJ, Sternberg C, Tolker-Nielsen T (2009) Insight into the microbial multicellular lifestyle via flow-cell technology and confocal microscopy. Cytometry 75A:90–103 44 L.K. Winkelströter et al.140. Pan Y, Breidt F Jr (2007) Enumeration of viable Listeria monocytogenes

cells by Real-Time PCR with propidium monoazide and ethidium monoazide in the presence of dead cells. Appl Environ Microbiol 73:8028–8031

141. Park SH, Cheon H-L, Park K-H, Chung M-S, Choi SH, Ryu S, Kang D-H (2012) Inactivation of biofilm cells of foodborne pathogen by aerosolized sanitizers. Int J Food Microbiol 154:130–134

142. Peeters E, Nelis HJ, Coenye T (2008) Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. J Microbiol Methods 72:157–165

143. Pérez-Osorio AC, Franklin MJ (2008) qRT-PCR of microbial biofilms. Cold Spring Harbor Protocols 3:1–8

144. Periasamy S, Joo H-S, Duong AC, Bach T-H L, Tan VY, Chatteriee SS, Cheung GYC, Otto M (2012) How Staphylococcus aureus biofilms develop their characteristic structure. PNAS 109:1281– 1286

145. Perrin C (2009) Nickel promotes biofilm formation by Escherichia coli K-12 strains that produce curli. Appl Environ Microbiol 75: 1723–1733

146. Pettit RK, Weber CA, Pettit GR (2009) Application of a high throughput Alamar blue biofilm susceptibility assay to Staphylococcus aureus biofilms. Ann Clin Microbiol Antimicrob 8:1–7

147. Pires D, Sillankorva S, Faustino A, Azeredo J (2011) Use of newly isolated phages for control of Pseudomonas aeruginosa PAO1 and ATCC 10145 biofilms. Res Microbiol 162:798–806

148. Poimenidou S, Belessi CA, Giaouris ED, Gounadaki AS, Nychas GJE, Skandamis PN (2009) Listeria monocytogenes attachment to and detachment from stainless steel surfaces in a simulated dairy processing environment. Appl Environ Microbiol 75:7182–7188

149. Ren D, Bedzyk LA, Thomas SM, Ye RW, Wood TK (2004) Gene expression in Escherichia coli biofilms. Appl Microbiol Biotechnol 64:515–524

150. Renner LD, Weibel DB (2011) Physicochemical regulation of biofilm formation. MRS Bull 36:347–355

151. Rieu A, Briandet R, Habimana O, Garmyn D, Guzzo J, Piveteau P (2008) Listeria monocytogenesEGD-e biofilms: no mushrooms but a network of knitted chains. Appl Environ Microbiol 74:4491–4497

152. Rode TM, Langsrud S, Holck A, Møretrø T (2007) Different patterns of biofilm formation in Staphylococcus aureus under

food-related stress conditions. Int J Food Microbiol 116:372–383

153. Saha R, Donofrio RS, Goeres DM, Bagley ST (2012) Rapid detection of rRNA group I pseudomonads in contaminated metalworking fluids and biofilm formation by fluorescent in situ hybridization. Appl Microbiol Biotechnol 94:799–808

154. Sauer K, Rickard AH, Davies DG (2007) Biofilms and biocomplexity. Microbe 2:347–353

155. Schleheck D, Barraud N, Klebensberger J, Webb JS, McDougald D, Rice SA, Kjelleberg S (2009) Pseudomonas aeruginosa PAO1 preferentially grows as aggregates in liquid batch cultures and disperses upon starvation. PLoS ONE 4:e5513

156. Seper A, Fengler VHI, Roier S, Wolinski H, Kohlwein SD, Bishop AL, Camilli A, Reidl J, Schild S (2011) Extracellular nucleases and extracellular DNA play important roles in Vibrio cholerae biofilm formation. Mol Microbiol 82:1015–1037

157. Shakerifard P, Gancel F, Jacquesb P, Faillec C (2009) Effect of different Bacillus subtilis lipopeptides on surface hydrophobicity and adhesion of Bacillus cereus 98/4 spores to stainless steel and Teflon. Biofouling 25:533–541

158. Sharma M, Anand SK (2002) Biofilms evaluation as an essential component of HACCP for food/dairy processing industry – a case. Food Control 13:469–477

159. Silley P, Forsythe S (1996) Impedance microbiology – a rapid change for microbiologists. J Appl Bacteriol 80:233–243

160. Silva S, Teixeira P, Oliveira R, Azeredo J (2008) Adhesion to and viability of Listeria monocytogeneson food contact surfaces. J Food Prot 71:1379–1385

161. Simões M, Simões LC, Vieira MJ (2010) A review of current and emergent biofilm control strategies. LWT Food Sci Technol 43: 573–583

162. Skillman LC, Sutherland IW, Jones MV, Goulsbra A (1998) Green fluorescent protein as a novel species-specific marker in enteric dual-species biofilms. Microbiology 144:2095–2101

163. Smith AW (2005) Biofilms and antibiotic therapy: is there a role for combating bacterial resistance by the use of novel drug delivery systems? Adv Drug Deliv Rev 57:1539–1550

164. Sofos JN (2009) Biofilms: our constant enemies. Food Safety Magazine 38:40–41

165. Sofos JN, Geornaras I (2010) Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of Escherichia coli O157:H7 in nonintact, and Listeria monocytogenesin ready-to-eat, meat products. Meat Sci 86:2–14

166. Spiers AJ, Rainey PB (2005) The Pseudomonas fluorescens SBW25 wrinkly spreader biofilm requires attachment factor, cellulose fibre and LPS interactions to maintain strength and integrity. Microbiology 151:2829–2839

167. Sriram MI, Kalishwaralal K, Deepak V, Gracerosepat R, Srisakthi K, Gurunathan S (2011) Biofilm inhibition and antimicrobial action of lipopeptide biosurfactant produced by heavy metal tolerant strain Bacillus cereus NK1. Colloids Surf B: Biointerfaces 85:74–181

168. Steenackers H, Hermans K, Vanderleyden J, De Keersmaecker SCJ (2012) Salmonella biofilms: an overview on occurrence, structure, regulation and eradication. Food Res Int 45:502–531

169. Stepanović S, Cirković I, Mijac V, Svabic-Vlahovic M (2003) Influence of the incubation temperature, atmosphere and dynamic conditions on biofilm formation by Salmonellaspp. Food Microbiol 20:339–343

170. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M (2000) A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods 40:175–179

171. Stewart PS, Murga R, Srinivasan R, Beer D (1995) Biofilm structural heterogeneity visualized by three microscopic methods. Water Res 29:2006–2009

172. Stier RF (2005) Beating back biofilms in food processing. Food Safety Magazine 11(1):31–34

173. Tang JN, Kang MS, Chen HC, Shi XM, Zhou R, Chen J, Wu DY (2011) The staphylococcal nuclease prevents biofilm formation in Staphylococcus aureus and other biofilm-forming bacteria. Science China 54:863–869

174. Teixeira P, Lima J, Azeredo J, Oliveira R (2008) Adhesion of Listeria monocytogenes to materials commonly found in domestic kitchens. Int J Food Sci Technol 43:1239–1244

175. Touhami A, Jericho MH, Boyd JM, Beveridge TJ (2006) Nanoscale characterization and determination of adhesion forces of Pseudomonas aeruginosa Pili by using atomic force microscopy. J Bacteriol 188:370–377

176. Trémoulet F, Duché O, Namane A, Martinie B (2002) Comparison of protein patterns of Listeria monocytogenes grown in biofilm or in planktonic mode by proteomic analysis. FEMS Microbiol Lett 210: 25–31

177. Unnerstad HE, Bannerman J, Bille M-L, Danielsson-Tham E, Waak W (1996) Prolonged contamination of a dairy with Listeria monocytogenes. Neth Milk Dairy J 50:493–499

178. Valle J, Re SD, Henry N, Fontaine T, Balestrino D, Latour-Lambert P, Ghigo J-M (2006) Broad-spectrum biofilm inhibition by a secreted bacterial polysaccharide. PNAS 103:12558–12563

179. Varga JJ, Therit B, Melville SB (2008) Type IV pili and the CcpA protein are needed for maximal biofilm formation by the grampositive anaerobic pathogen Clostridium perfringens. Infect Immun 76:4944–4951

180. Verghese B, Lok M, Wen J, Alessandria V, Chen Y, Kathariou S, Knabel S (2011) ComK prophage junction fragments as markers for Listeria monocytogenes genotypes unique to individual meat and Microbial Biofilms of Importance 45 poultry processing plants and a model for rapid niche-specific adaptation, biofilm formation and persistence. Appl Environ

Microbiol 77:3279–3292

181. Verran J, Packer A, Kelly P, Whitehead KA (2010) The retention of bacteria on hygienic surfaces presenting scratches of microbial dimensions. Lett Appl Microbiol 50:258–263

182. Vilain S, Pretorius JM, Theron J, Brözel VS (2009) DNA as an adhesion: Bacillus cereus requires extracellular DNA to form biofilms. Appl Environ Microbiol 75:2861–2868

183. Waite RD, Papakonstantinopoulou A, Littler E, Curtis MA (2005) Transcriptome analysis of Pseudomonas aeruginosa growth: comparison of gene expression in planktonic cultures and developing and mature biofilms. J Bacteriol 187:6571–6576

184. Wang Q, Frye JG, McClelland M, Harshey RM (2004) Gene expression patterns during swarming in Salmonella typhimurium: gene specific to surface growth and putative new motility and pathogenicity genes. Molecula Microbiology 52:169–187

185. Wang X, Preston JF III, Romeo T (2004) The pgaABCD locus of Escherichia coli promotes the synthesis of a polysaccharide adhesion required for biofilm formation. J Bacteriol 186:2724–2734

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

187. Wijman JGE, Leeuw PPLA, Moezelaar R, Zwietering MH, Abee T (2007) Air–liquid interface biofilms of Bacillus

cereus: formation, sporulation, and dispersion. Appl Environ Microbiol 73:1481–1488

188. Winkelströter LK, Gomes BC, Thomaz MRS, Souza VM, De Martinis ECP (2011) Lactobacillus sakei 1 and its bacteriocin

influence adhesion of Listeria monocytogenes on stainless steel surface. Food Control 22:1404–1407

189. Wirtanen G, Salo S, Helander IM, Mattila-Sandholm T (2001) Microbiological methods for testing disinfectant efficiency on Pseudomonas biofilm. Colloids Surf B: Biointerfaces 20:37–50

190. Wright CJ, Shah MK, Powell LC, Armstrong I (2010) Application of AFM from microbial cell to biofilm. Scanning 32:134–149

191. Ye RW, Wang T, Bedzyk L, Croker KM (2001) Applications of DNA microarrays in microbial systems. J Microbiol Methods 47: 257–272

192. Yeom J, Lee Y, Park W (2012) Effects of non-ionic solute stresses on biofilm formation and lipopolysaccharide production in Escherichia coli O157:H7. Res Microbiol 163:258–267

193. Zhu X, Liu W, Lametsch R, Aarestrup F, Shi C, She Q, Shi X, KnØchel (2011) Phenotypic, proteomic, and genomic characterization of a putative ABC-transporter permease involved in Listeria monocytogenes biofilm formation. Foodborne Pathogens and Disease 8:495–501

194. Zottola EA, Sasahara KC (1994) Microbial biofilms in the food processing industry—should they be a concern? Int J Food Microbiol 23:125–148 46 L