# Pernicious Attitude of Microbial Biofilms in Agri-Farm Industries

Subjects: Food Science & Technology

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Biofilm is a complex matrix made up of extracellular polysaccharides, DNA, and proteins that protect bacteria against physical, chemical, and biological stresses and allow them to survive in harsh environments. Safe and healthy foods are mandatory for saving lives. However, foods can be contaminated by pathogenic microorganisms at any stage from farm to fork. The contaminated foods allow pathogenic microorganisms to form biofilms and convert the foods into stigmatized poison for consumers. Biofilm formation by pathogenic microorganisms in agri-farm industries is still poorly understood and intricate to control. In biofilms, pathogenic bacteria are dwelling in a complex manner and share their genetic and physicochemical properties making them resistant to common antimicrobial agents. Therefore, finding the appropriate antibiofilm approaches is necessary to inhibit and eradicate the mature biofilms from foods and food processing surfaces. Advanced studies have already established several emerging antibiofilm approaches including plant- and microbe-derived biological agents, and they proved their efficacy against a broad-spectrum of foodborne pathogens.

pathogenic biofilm | foodborne pathogen | food safety | antibiofilm control | green approach

## 1. Introduction

On earth, nearly 99% of bacterial organisms are likely to dwell in a complex community called a biofilm [1]. In a biofilm, a mixture of communities of mono- and mixed-bacterial species are formed in a heterologous architecture surrounded by extracellular polymeric secretions (EPS) that are comprised of environmental DNA (eDNA), polysaccharides, lipopeptides, and proteins. The secreted EPS matrix helps to defend bacteria against physical, chemical, and biological stresses by improving their capability to retain nutrients and water from their surroundings, which allows them to survive in harsh environmental conditions [2]. The accumulation of a bacterial community and the establishment of biofilms may depend on the locations and the favorable substratum influenced by environmental factors (e.g., temperature, nutrients, community interactions, and osmolarity) [3]. Biofilms are commonly found on moisture-laden surfaces, such as food, conveyer belts, processing instruments, water systems, and packaging lines [4]. Bacterial biofilms are established with the preparation of favorable bases (e.g., biotic and abiotic surfaces) after taking up organic or inorganic molecules (e.g., polysaccharides, glycoproteins, and lipids) [5]. Following the bacterial attachment on surfaces, the community members habitually interconnect by cellular signaling systems, such as quorum-sensing (QS) [6]. The formation and development of bacterial biofilms is a sequential process (**Figure 1**) consisting of (i) initial attachment, (ii) irreversible attachment and cell-to-cell adhesion, (iii) early development of biofilm proliferation, (iv) maturation, and (v) dispersion [7].

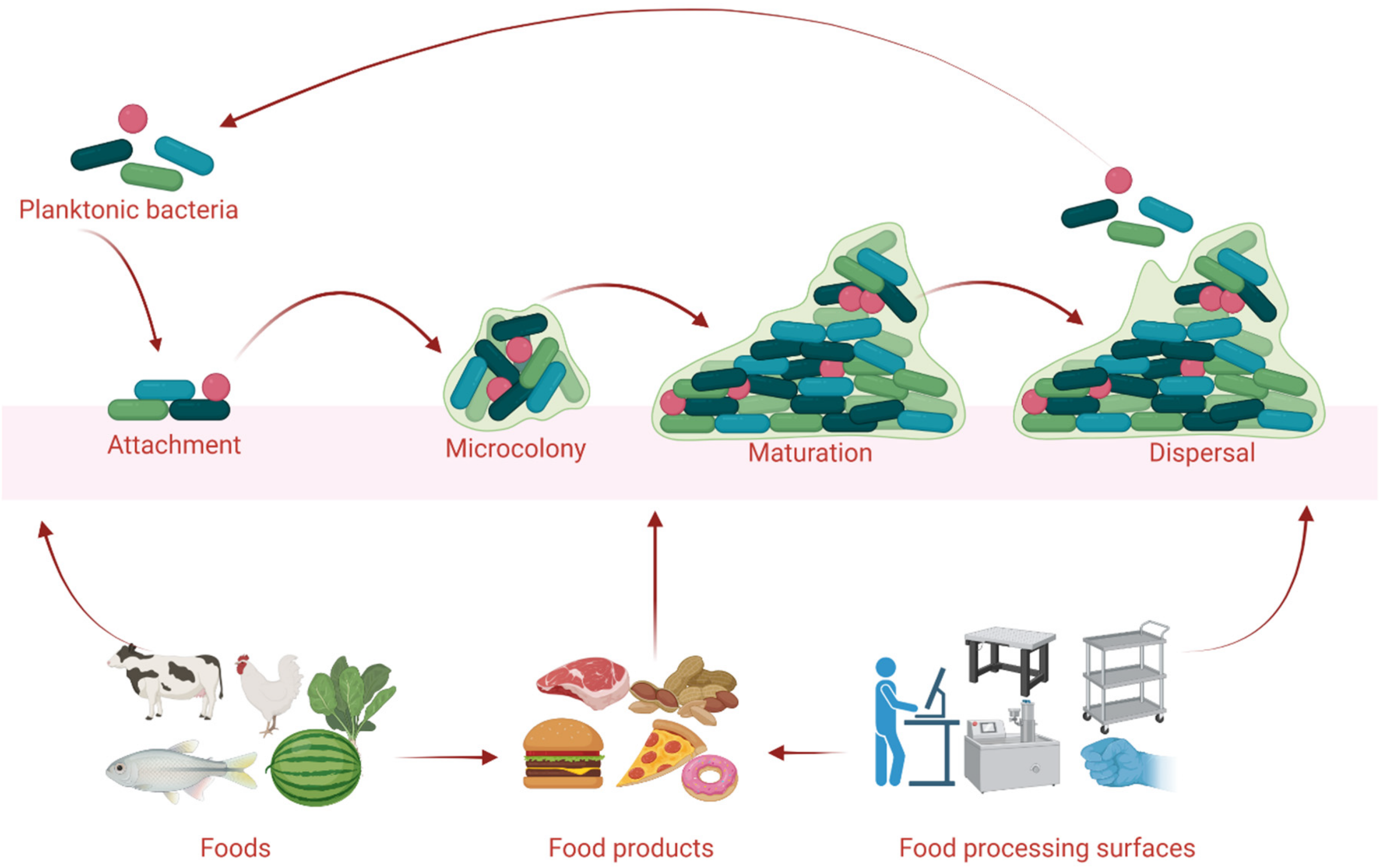


**Figure 1.** The formation and development of bacterial biofilms in foods and food processing surfaces in agri-farm industries. The figure was illustrated using BioRender.com (accessed on 22 July 2022).

Biofilm formation is a major problem in different agri-farm industries, including dairy, meat, aquatic, and agricultural product processing plants [8][9][10]. It has been estimated that more than 31 known and countless unknown microorganisms are responsible for foodborne illness worldwide and approximately 66% of human diseases are associated solely with pathogenic bacteria [11][12]. The National Institute of Health (NIH) has reported that more than 250 identified and many unidentified diseases are associated with the consumption of unsafe foods, where bacterial biofilms are mostly responsible for ~65% of the microbial and 80% of the chronic infections of humans [13][14]. Spoilage and pathogenic bacteria colonize inside the blending tanks, vats, and piping systems in processing plants; therefore, the formed biofilms threaten the safety and quality of food products. Several factors have also accounted as an influencer on the development of microbial biofilms, in particular foods and food processing plants, such as bacterial strain specificity and their suitable growth conditions (e.g., water, required nutrients, pH, temperature) [15][16]. The majority of previous research to date focuses on planktonic bacteria properties and control. However, biofilm formation by bacteria or other microbes is more resistant (10–10,000 times) to antimicrobial than the planktonic state [13]. They have a barrier to antimicrobial agents that prevent or reduce contact [7][17].

# 2. Microbial Biofilms in Food Processing Industries

Despite the advantageous behavior of beneficiary microorganisms, the formation of pathogenic biofilms by foodborne microorganisms on food and food processing surfaces could contaminate the raw materials and the processing lines of food products. However, pathogenic contamination that leads to the formation of undesirable biofilms in food industries is still poorly understood and hard to control. Therefore, drawing out the proper outlines of the pathogenic biofilm formation and finding the appropriate remedy to inhibit it in the food industry is crucial.

## 2.1. Biofilm Associated Problems in the Dairy Industry

The presence of foodborne microorganisms in raw products and processing plants, leading to the formation of bacterial biofilms is one of the critical problems facing the dairy industry [10][18]. Following the attachment, bacterial colonies generally persist on the foods and food-processing surfaces, such as processing tanks, vats, and pipelines which continue their involvement in the dairy product contamination and compromise the product quality, economic defeat, and public health safety worldwide [17]. In the dairy processing industry, different classes of microorganisms are involved, based on their advantageous and disadvantageous role of efficacy [7]. A wide variety of thermophilic and cryophilic foodborne microorganisms can occupy and endure in any stage from processing to packaging, due to the inadequate pasteurization procedure and handling of end dairy products [19]. For instance, several pathogenic microbial species (e.g., *Bacillus*, *Citrobacter*, *Enterobacter*, *Pseudomonas*, *Raoultella*, and *Klebsiella* spp.) have been detected from storage tanks and processing pipelines wastewater systems in dairy milk powder processing plants [20]. These acute biofilm formers can persist and induce the formation of bacterial biofilm on food surfaces, leading to taint the milk storage systems by colonization, and spores may remain on the packing surfaces of the end products [17]. Moreover, cryophilic bacteria, such as *Pseudomonas fluorescens*, *P. putrefaciens*, and *Listeria monocytogenes,* could make milk and other dairy products difficult to store since they can thrive at cooling temperatures [21]. Heat-stable lipolytic and proteolytic enzymes produced by pathogenic microorganisms (e.g., *Pseudomonas and Serratia* spp.) have also been accounted as milk and dairy product spoilers, by reducing the product shelf-life and inducing the strong off-flavors, such as bitterness, rancidity, or aged taste [19].

### Biofilm Control Strategies in the Dairy Industry

Foodborne pathogenic bacteria contamination in dairy raw materials is particularly leading to the formation of biofilms on processing equipment and the surrounding environmental surfaces of dairy plants. Therefore, finding a suitable antimicrobial agent is crucial and the antimicrobial strategies required to consider before applying to the dairy processing plants, include surface chemical modification, surface treatment by means of antimicrobials, manufacturing process optimization, and in-depth knowledge of dairy processing machinery and their cleaning procedures for subsequent bacterial biofilm inhibition [10][21]. In the dairy industry, the standard cleaning practice has an imperative role in controlling foodborne pathogenic bacterial growth and inhibiting biofilm formation in dairy manufacturing equipment [22]. For instance, clean-in-place (CIP) in the dairy plants primarily removes fouling materials and the procedure includes washing milk processing lines with chemicals for cleaning and sanitation, and using more antimicrobial materials for an improved efficiency [23]. The first and most crucial step in improving the sanitation of the processing equipment might significantly influence the quality of the end products. A wide range of sanitizers, such as surfactants, enzymes, and alkali compounds are used in dairy processing industries for eliminating contaminants, by reducing the surface tension, emulsifying fats, and denaturing proteins [19][21]. For instance, Toté et al. observed that chemical disinfectants, such as sodium hypochlorite, hydrogen peroxide, peracetic acid, and isopropanol, could successfully inhibit the bacterial biofilm formation and reduce the viable cells of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in food contact surfaces [24]. Moreover, cell-free supernatants from probiotic bacteria (*Lactobacillus sakei* D.7 and *Lactobacillus plantarum* I.60) have exhibited the excellent eradication efficacy of *L. monocytogenes* biofilm formed in whole milk [10].

## 2.2. Biofilm-Associated Problems in the Meat Processing Industry

The adhesion and formation of mature biofilms by foodborne microorganisms during the manufacturing and handling of fresh meats, have remained a serious concern for consumer health and food safety. Bacterial populations can contaminate non-adulterated carcasses and fresh meat products by spreading through aerosols or direct contact with the surface of slaughter- and manufacturing-related equipment. In the meat processing plant, numerous species of bacteria, including *Escherichia*, *Salmonella*, *Staphylococcus*, *Bacillus*, and *Pseudomonas* spp. could take place to form pathogenic biofilms that primarily contribute to the spoilage of meat products and food-associated infections in the consumers [25]. For instance, beef carcass contamination with *E. coli* O157: H7 may occur while being slaughtered, dressed, chilled, and/or trimmed in the beef processing plant, at a wide range of temperatures [26]. Pathogenic microorganisms have the potential to attach to meat and meat processing surfaces and the expressed specific virulence factors, including adhesins, flagella, curli, fimbria, and enterocyte locus, which play vital roles to initiate and form pathogenic biofilms. Habimana et al. have reported that *E. coli* could be influenced by *Acinetobacter calcoaceticus* and form mixed-bacterial biofilms in the meat processing plant [27]. Hathroubi et al. revealed that surface polysaccharide poly-N-acetyl glucosamine (PGA) could influence the pathogens, such as *A. pleuropneumoniae*, *E. coli*, and *S. aureus* for the antibiotic tolerance and formation of biofilms on meat products [28]. The sheep–goat chain plays an important and significant role in the socioeconomic development of certain countries, mainly in poor and semi-arid zones [25]. Among other probable foodborne pathogens, a staphylococcal contamination was widely reported in bulk goat milk [29]. In sheep and goat meat processing plants, *S. aureus*, coagulase-negative staphylococci (CoNS), *Bordetella parapertussis*, *Bacillus* spp., *Histophilus somni*, and *Pasteurella multocida* were identified as the major pathogenic biofilm formers [30].

Poultry products, such as poultry meats and eggs, are considered as an enriched source of nutrients (e.g., protein) with less fat and have become popular with consumers due to their availability and cheaper prices worldwide [31]. However, poultry products could be contaminated by various foodborne microorganisms, principally, by *Salmonella* and *Campylobacter* spp. [32]. According to the Centers for Disease Control and Prevention (CDC), the pathogenic contamination of boiler meats and eggs could be initiated from numerous sources, including the drinking water supply system on farms and poultry feeds, and causing about 96 million cases of foodborne gastroenteritis illnesses each year, globally [33]. Gazal et al. isolated and identified 117 strains of *E. coli,* after an investigation of commercial chicken processing plants [34]. Among the isolates, 66% of the strains were extended-spectrum *β*-lactamase and AmpC-like enzyme producers, which can effectively degrade the *β*-lactam class of antibiotics (e.g., monobactams and cephalosporins). The frequency of poultry product contamination may rise, due to inadequate knowledge about poultry slaughtering, faulty cutting, and insufficient hygiene practices during production and processing. For instance, *Listeria* spp. was identified from broiler wing meat samples collected from the local market in Hatay province in Turkey [35]. Heidemann et al. have reported about the pathogenic microbial-associated infection pododermatitis in chicken farms and identified 106 bacterial isolates, including *E. coli*, *S. aureus*, *Staphylococcus hyicus*, *Enterococcus faecalis*, *Aerococcus urinaeequi*, *Gallibacterium anatis*, and *Trueperella pyogenes* from the table egg layers [36]. Moreover, using rubber fingers for removing the feathers from carcasses could be considered a potential source of product contamination by pathogenic microorganisms in commercial poultry processing plants [32].

## 2.3. Biofilm Associated Problems in the Aquatic Industry

Fishing is one of the oldest activities carried out by humans, dating back to prehistoric times and aquacultures have a high demand due to their important role in the world economy, particularly in coastal communities and developing countries. To date, approximately 0.6 billion people (10% of the total world population) primarily rely on the aquatic biodiversity for their livelihood and subsistence (Food and Agriculture Organization [FAO]) [37]. From a world hunger and nutrition standpoint, aquatic foods are considered as the major protein source and could be the best alternative to animal-derived proteins [38]. Aquatic foods can be contaminated and decay rapidly during any stage of the production and distribution process, due to the biochemical degradation and the presence of pathogenic bacteria on their surfaces after capture. Zoonotic bacterial species, such as *Staphylococcus* spp., *Pseudomonas* spp., *Listeria* spp., *Salmonella* spp., *Vibrio* spp., *Aeromonas hydrophila*, and *E. coli* are primarily responsible for the biofilm formation and aquatic-associated disease outbreaks worldwide [3]. The failure of proper handling and inadequate sanitation procedures in aquatic processing facilities is deemed to be the persistence of aquatic-associated bacteria in aquatic foods and food contact surfaces [39]. Fresh and salt water fishes become colonized with pathogens in their surfaces or inner organs from polluted aquatic environments, that lead to the formation of bacterial biofilms [40]. For instance, *S. aureus* is repeatedly detected in fishery products, which is responsible for the foodborne intoxications (e.g., staphylococcal enterotoxins [SEs]) in humans worldwide. To date, a total of 23 SEs-associated genes (e.g., *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *she*, *sei*, *selj*, *sek*, *sel*, *sem*, *sen*, *seo*, *seq*, *sep*, *ser*, *ses*, *set*, *selu*, *selu2*, *selv*, and *selx1*) were reported after the screening of 1545 *Staphylococcus* spp. and 97% of *S. aureus* having one or more enterotoxigenic (ET) genes in their genome [41]. Ham et al. identified the presence of *Staphylococcus* spp. in 33.8% dried seasoned fish products, among 210 samples, which were collected from the South Korean retail market [42]. A study conducted by Moon et al. exhibited that ET gene *se*-carrying *S. aureus* could cross-contaminate aquatic food products and facilitate the biofilm formation under refrigerated conditions [43]. The thermal- and protease-resistance nature of ET produced by *Staphylococcus* spp. could retain their emetic activity even after marine food processing and enhance the risk of intoxication [20]. Several studies have also reported the persistence of *Leptospira* spp., *Yersinia* spp., *L. monocytogenes*, *Aeromonas hydrophila*, and *Francisella tularensis* in aquatic food processing facilities, due to inadequate handling and ineffective sanitizing procedures [39]. *L. monocytogenes* serotypes 1/2a, 1/2b, 1/2c, and 4b are frequently found in both fisher products and fish-processing contact surfaces and are considered the causative agent of human listeriosis (e.g., febrile gastroenteritis and systemic infections). Skowron et al. identified 237 *L. monocytogenes* isolates after investigating the fish products and fish-processing surfaces and found a total number of 161 genetically dissimilar strains, via the pulsed-field gene electrophoresis method [44].

## 2.4. Biofilm Associated Problems in the Agricultural Industry

Plant-microbe interactions have a necessary influence on plant nutrition, growth, biocontrol, and stress alleviation. The equilibrium of soil nutrients is also dependent on the interactions via physical, chemical, and biological properties persuaded by biogeochemical cycles in the soil [45]. The presence of pathogenic bacteria in the environment (e.g., soil and water) might adhere and colonize plant surfaces during pre-harvesting (propagation) and post-harvesting (processing), which can lead to biofilm-associated problems in the agricultural industry. The colonization of pathogenic microorganisms could occur on plants via seeds, roots, leaves, stems, and vascular tissues (i.e., xylem and phloem). Following the adhesion of pathogenic microorganisms on plant tissue surfaces, the microcolony formation of pathogenic cells turns into massive biofilm structures via the plant-microbe interaction (e.g., pathogenesis, mutualism, or commensalism) [46]. The nutrient and water accessibility in plant tissue surfaces, in particular the proclivity of the microbial colonization, thus manipulates the formation of pathogenic cell clusters in biofilms [7]. Bacteria-associated microbial hazards are mostly observed in freshly produced agriculture products and are responsible for foodborne diseases worldwide [47][48]. To date, many outbreaks have been associated with the consumption of freshly produced agriculture products, including carrots, lettuce, cucumbers, onions, spinach, and tomatoes due to the surface colonization by the biofilm-forming pathogens (e.g., *Salmonella* spp., *Campylobacter* spp., *Vibrio* spp., *Shigella* spp., *Clostridium* spp., *L. monocytogenes*, *E. coli*, *Aeromonas hydrophila*, and *Bacillus cereus*) [45][49]. The use of contaminated soil and water for plant irrigation could act as a reservoir and route of pathogenic microbial transmission that causes foodborne illness to consumers [13]. The diversity of multicellular assemblies of microbes on plant surfaces varied, in terms of morphology from microcolony formations, aggregates, and clusters in specific or scattered locations. Additionally, numerous factors (e.g., age of biofilms, nutrient levels, oxygen levels, EPS, aggregation, waste product accumulation, mechanical signals, host-derived signals, antimicrobials, biocides, metal ion concentrations, and plant volatiles) have significant roles during the plant-associated biofilm formation [46]. Recently, the CDC has reported on the increments of fresh produce-associated disease outbreaks worldwide in the last decade compared to other food products [33]. The investigation carried out on fresh produce-associated human illness in European countries reported that *Salmonella* spp. had the highest presence (0.1–2.3%) in fresh-cut fruits and vegetables [50]. The European Food Safety Authority (EFSA) reported that more than 10% of outbreaks were linked with freshly produced food products from 2007 to 2011, which accounted for the hospitalization of approximately 35% of people and 46% of deaths in Europe [51]. Recently, *Salmonella* spp. has been reported as the major zoonotic bacteria found in fresh papayas and pre-cut melons, which caused about 188 cases in different states of the USA, in 2019 [49]. However, fresh-produce-associated outbreaks are mostly reported in developed countries (e.g., Canada, USA, Australia, and the European continent), compared to developing or underdeveloped countries, due to their insufficient technology for the surveillance of foodborne-associated diseases.

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