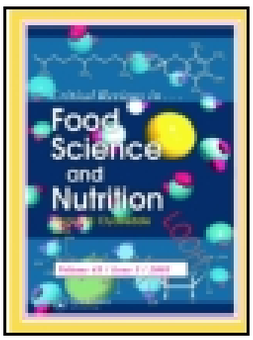

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

Mixed-species biofilms in the food industry: Current knowledge and novel control strategies

Lei Yuan, Mads Frederik Hansen, Henriette Lyng Røder, Ni Wang, Mette Burmølle & Guoqing He

To cite this article: Lei Yuan, Mads Frederik Hansen, Henriette Lyng Røder, Ni Wang, Mette Burmølle & Guoqing He (2019): Mixed-species biofilms in the food industry: Current knowledge and novel control strategies, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2019.1632790

To link to this article: https://doi.org/10.1080/10408398.2019.1632790

Published online: 01 Jul 2019.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2019.1632790

Taylor & Francis
Taylor & Francis Group

REVIEW

Mixed-species biofilms in the food industry: Current knowledge and novel control strategies

Lei Yuan[a,b], Mads Frederik Hansen[b], Henriette Lyng Røder[b], Ni Wang[a], Mette Burmølle[b], and Guoqing He[a]

[a]College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, China; [b]Section of Microbiology, Department of Biology, University of Copenhagen, Copenhagen, Denmark

ABSTRACT

Attachment of microorganisms to food contact surfaces and the subsequent formation of biofilms may cause equipment damage, food spoilage and even diseases. Mixed-species biofilms are ubiquitous in the food industry and they generally exhibit higher resistance to disinfectants and antimicrobials compared to single-species biofilms. The physiology and metabolic activity of microorganisms in mixed-species biofilms are however rather complicated to study, and despite targeted research efforts, the potential role of mixed-species biofilms in food industry is still rather unexplored. In this review, we summarize recent studies in the context of bacterial social interactions in mixed-species biofilms, resistance to disinfectants, detection methods, and potential novel strategies to control the formation of mixed-species biofilms for enhanced food safety and food quality.

KEYWORDS

Mixed-species biofilms; interspecies interactions; biofilm control strategies; food safety; food quality

Introduction

Bacteria frequently adhere to surfaces and form spatially-organized communities within a self-produced matrix composed of extracellular polymeric substances (EPS), known as biofilms (Flemming and Wingender 2010). Biofilms represent serious challenges to the food industry since they enable bacteria to adhere to various kinds of surfaces, including plastic, polypropylene, stainless steel, glass, rubber, and even the food products themselves within few minutes, followed by the formation of mature biofilms within several days or even hours (Hall-Stoodley, Costerton, and Stoodley 2004). Due to the physiological changes and the protective barrier mediated by the biofilm matrix, this sessile life mode has represented an outstanding survival strategy for microorganisms since ancient times, as it protects against stressful environmental conditions that are commonly encountered by bacteria in natural and man-made habitats, including those present in food processing facilities (Acker, Dijck, and Coenye 2014; Alvarez-Ordóñez et al. 2019). Thus, biofilms have notoriously been identified as being responsible for equipment damage, increased energy costs, food spoilage and diseases (Alvarez-Ordóñez et al. 2019; Brooks and Flint 2008; Møretrø and Langsrud 2017; Yuan et al. 2018b).

Although mixed-species biofilms represent the most frequent form of contamination in the food industry, the majority of our knowledge regarding biofilm formation is based on studies of single-species biofilms (Srey, Jahid, and Ha 2013; Galié et al. 2018). These studies have generated a substantial amount of information, but do not necessarily reflect the potential of mixed-species biofilms, as interspecies interactions facilitate bacterial properties different from those of single-species biofilms (Burmølle et al. 2014). The combination of advanced techniques and increased acknowledgment of the importance of interspecific interactions in complex communities in recent years has however gradually shifted focus towards exploring the complexity and interactions in mixed-species consortia. The importance of mixed-species interactions in biofilms in the food sector is further accentuated by a range of recent studies (Table 1), involving dairy processing, brewing, fermentation, foodservice facilities, fresh produce, fish processing, and meat processing. More importantly, most mixed-species biofilms have been shown to enhance the resistance of residing cells to disinfectants and their persistence on food contact surfaces compared to mono-species biofilms. This emphasizes the importance and relevance for further studies on complex consortia in the context of food industry in order to identify effective solutions to avoid cross-contamination (Ibusquiza et al. 2012; Pang, Yang, and Yuk 2017; Pang and Yuk 2018). The objective of this review is to provide an overview of the current knowledge related to microbial interactions in these complex communities, especially focusing on their resistance to disinfectants and sanitizers. We will describe useful techniques for studying mixed-species biofilms, and discuss the current and innovative control strategies that have been used to combat the challenges caused by biofilms.

CONTACT Mette Burmølle burmolle@bio.ku.dk University of Copenhagen, Universitetsparken 15, Copenhagen Ø, 2100, Denmark; Guoqing He gqhe@zju.edu.cn Zhejiang University, 866 Yuhangtang Road, Hangzhou, 310058, China.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bfsn.

Table 1. Mixed-species consortia found in food industry.

Locations	Predominated microorganisms at species or genus level	Differentiation methods	References
Whey reverse osmosis membranes	*Lactobacillus*, *Lactococcus*, Coliform, *Pseudomonas* and *Staphylococcus*	16S rDNA sequencing	Anand, Hassan, and Avadhanula (2012)
Spiral-wound membranes in dairy plants	*Lactococcus*, *Arthrobacter*, *Streptococcus*, *Enterococcus*, *Methylobacterium*, *Acinetobacter*, *Cronobacter* and *Klebsiella*	High-throughput sequencing	Chamberland et al. (2017)
Crevices of cleaned devices from a milk processing plant	*Pseudomonas*, *Staphylococcus*, *Serratia*, *Stenotrophomonas* and *Alcaligenes*	16S rDNA sequencing	Cleto et al. (2012)
Stainless steel pipes of a milk-processing dairy plant	*Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus hominis*	Amplified ribosomal DNA restriction analysis and 16S rDNA sequencing	Cherif-Antar et al. (2016)
Sprinklers from dairy farm cooling systems	*Paracoccus*, *Methyloversatilis*, *Brevundimonas*, *Porphyrobacter*, Gp4, *Mycobacterium*, *Hyphomicrobium*, *Corynebacterium* and *Clostridium*	Pyrosequencing and 16S rRNA sequencing	Shpigel et al. (2015)
Eleven different sites along the processing lines of a milk powder processing	*Acinetobacter*, *Clostridium*, *Enterobacter*, *Microbacterium*, *Micrococcus*, *Moraxella*, *Pseudomonas*, *Serratia* and *Staphylococcus*	16S rDNA sequencing	Zou and Liu (2018)
The surface of vinegar elaborated in wood barrels	*Ameyamaea*, *Gluconacetobacter*, *Bacillus* and *Komagataeibacter*	PCR-DGGE, pyrosequencing, PCR-RFLP and 16s rRNA sequencing	Valera et al. (2015)
A primary treated dairy wastewater system in a dairy milk powder plant	*Pseudomonas*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Bacillus* and *Raoultella*	Next-generation genomic sequencing	Dixon et al. (2018)
Various surfaces of a brewery processing plant	*Lactobacillus*, *Pediococcus*, *Enterobacter*, *Bacillus*, *Pseudomonas*, *Acinetobacter* and *Sphingomonas*	16S rDNA sequencing	Maifreni et al. (2015)
Floor drains in cooling chamber, and cutting, washing and processing areas of cheese plants	*Leuconostoc citreum*, *Pseudomonas mucidolens*, *Lactococcus lactis*, *Pseudomonas fragi* and *Acetobacter tropicalis*	Pyrosequencing	Dzieciol et al. (2016)
Multiple food contact and noncontact surfaces in fresh produce processing plants	*Rahnella aquatilis*, *Pseudomonas fluorescens*, *Ralstonia insidiosa*, *Bacillus pumilus* and *Enterobacter cloacae*	The Biolog Gen III microbial identification system	Liu et al. (2013)
Dairy reverse osmosis and ultrafiltration membranes	*Chryseobacterium indologenes*, *Bacillus firmus*, *Lactococcus lactis* ssp *cremoris*, *Klebsiella oxytoca*, *Enterobacter sakazakii*, *Lactobacillus* spp., *Bacillus licheniformis*, *P. fluorescens* and *Blastoschizomyces capitatus*	The API culture identification system	Tang et al. (2009)
Stainless steel pipeline in collect center of raw bovine milk production	*L. lactis*, *Staphylococcus xylosus*, *B. cereus* and *Candida albicans*	16S rDNA gene sequencing	Ksontini, Kachouri, and Hamdi (2013)
Different surfaces of equipment or floors in a meat-processing plant	*Bacillus*, *Staphylococcus*, *Micrococcus*, *Corynebacterium* and *Pseudomonas*	16S rRNA sequencing	Marouani-Gadri, Augier, and Carpentier (2009)
Conveyor belts in meat processing plants	*Pseudomonas*, *Acinetobacter*, *Microbacterium*, *Sphingomonas* and *Epilithonimonas*	16S rRNA sequencing	Fagerlund et al. (2017)
The wasted coffee tray leach	*Enterococcus*, *Pseudomonas*, *Stenotrophomonas*, *Sphingobacterium* and *Acinetobacter*	16S rRNA gene sequencing	Vilanova, Iglesias, and Porcar (2015)
Conveyors in the salmon-processing environment after cleaning and disinfection	*Pseudomonas*, *Acinetobacter*, *Serratia* and *Rhodococcus*	16S rRNA sequencing	Langsrud et al. (2016)
Twenty-three areas in a cafeteria kitchen	*Bacillus*, *Acinetobacter*, *Kocuria* and *Staphylococcus*	16S rRNA sequencing	Lim et al. (2017)
Several surfaces in seven food companies after cleaning and disinfection	*Pseudomonas*, *Microbacterium*, *Stenotrophomonas*, *Staphylococcus*, and *Streptococcus*	16S rRNA sequencing	Maes et al. (2019)

Biofilm formation in food processing environments

Modern food processing lines provide an environment well-suited for biofilm formation on food contact surfaces, mainly due to the complexity of processing facilities, lengthy production cycles, mass production of products, and the vast areas for biofilm development (Lindsay and von Holy, 2006). Thus, the majority of foodborne bacteria can attach to contact surfaces encountered in these areas, and lead to an increased risk of bacterial foodborne diseases. For example, it is estimated that 80% of bacterial infections in the United States is directly linked to foodborne pathogens residing in biofilms (Srey, Jahid, and Ha 2013). Several studies have described the ability of foodborne pathogens to attach to different food contact surfaces and form biofilms, including *Escherichia coli* O157:H7 (Al-Shabib et al. 2017), *Staphylococcus aureus* (Millezi et al. 2012), *Listeria monocytogenes* (Alavi and Hansen 2013), *Campylobacter jejuni* (Ica et al. 2012), *Vibrio parahaemolyticus* (Mizan et al. 2018), and *Salmonella enterica* (Liu et al. 2016a). These pathogens are well-known causative agents of hemorrhagic colitis and hemolytic uremic syndrome (Rangel et al. 2005), listeriotic (Buchanan et al. 2017), and bacterial gastroenteritis (Skarp, Hänninen, and Rautelin 2016; Luo et al. 2018; Li et al. 2019). Also, spoilage-associated microorganisms belonging to the genera of *Pseudomonas*, *Acinetobacter*, *Serratia*, *Shewanella*, *Chryseobacterium*, *Flavobacterium*, and *Enterococcus*, have been described to attach to food contact surfaces and form biofilms (Yuan et al. 2018a; Vilanova, Iglesias, and Porcar 2015). Particularly, good biofilm

formers, such as *Pseudomonas* and *Acinetobacter*, have been found to include poor or non-biofilm producers and increase the total biofilm formation of the consortia (Ibusquiza et al. 2012; Zupančič et al. 2018).

Factors that affect microbial attachment to surfaces

The formation of mixed-species biofilm is highly dynamic and depends on the properties of the attachment surface (Tang et al. 2011), the food matrix constituents (Van Houdt and Michiels 2010), environmental factors (Govaert et al. 2018; Ibusquiza et al. 2015), and the bacterial cells involved (Makovcova et al. 2017; Yuan et al. 2018a).

The properties of the attachment surface, such as electrostatic charge, hydrophobicity, interface roughness and topography, affect biofilm formation and thus determine the overall hygienic status of surfaces (Araújo et al. 2010; Tang et al. 2011). However, the specific effect of these parameters varies greatly under different experimental conditions. Some studies have shown that bacterial attachment will occur most readily on rougher surfaces (Tang et al. 2011; Dhowlaghar et al. 2018), while others found no relationship between roughness and bacterial attachment (Jindal et al. 2018). Similarly, there is a tendency for hydrophobic surfaces to attract more bacteria, but opposing results were obtained from studies testing the effect of hydrophobicity (Gomes et al. 2015; Veluz et al. 2012), although some studies proved that hydrophilic surfaces facilitate more bacterial adherence than hydrophobic equivalents (Dhowlaghar et al. 2018; Jindal et al. 2018). The lack of consistent conclusions can be explained by the use of different methods and bacterial strains, and also by the fact multiple factors are likely to determine the overall attachment. The most common food contact material used in the food industry is stainless steel type 304, because it is chemically inert at a variety of processing temperatures, easy to clean, and highly resistant to corrosion. However, due to the continuous use, the topography of this material generally contains cracks and crevices, which protect the bacteria from mechanical cleaning methods and sanitizing treatments.

Bacterial attachment is also highly affected by food matrix constituents in food processing environments (Iñiguez-Moreno, Gutiérrez-Lomelí, and Avila-Novoa 2019). For example, food residues such as milk and meat exudate that enriching in proteins, fats, and carbohydrates, promote the growth and multiplication of microorganisms, and favored the dual-species biofilm formation by *E. coli* and *S. aureus* (Dutra et al. 2018). Milk lactose increased biofilm formation by both *Bacillus subtilis*, through activation of the LuxS-mediated quorum-sensing system (Duanis-Assaf et al. 2016), and *S. aureus*, through promoted polysaccharide intercellular adhesion (Xue, Chen, and Shang 2014). High concentrations of free Ca^{2+} and Mg^{2+} in milk resulted in enhanced biofilm formation by *Geobacillus* spp. (Somerton et al. 2015).

The properties of the microbial cells, particularly the hydrophobicity, appendages (e.g. flagella, pili, fimbriae), the cellular membrane components (e.g. protein and lipopolysaccharide), and the EPS secreted by bacteria also play key roles in promoting biofilm formation (Van Houdt and Michiels 2010). Variances in biofilm-forming capacity among species or strains from different serotypes and genotypes have been described, suggesting the evolution of enhanced biofilm formation from different genetic backgrounds (Yuan et al. 2018a; Wang, Kalchayanand, and Bono 2015). In a mixed microbial community, different species can also affect each other, resulting in co-colonization of certain species, which will be discussed in the section of 'Bacterial social interactions in mixed-species biofilms'. Furthermore, environmental conditions, such as nutrient composition, pH, temperature, O_2 and shear forces, also play important roles in the process of biofilm formation (Govaert et al. 2018; Ibusquiza et al. 2015; Chamberland et al. 2019).

The complexity of the surface and microbial interactions thus makes it impossible to predict biofilm formation based on any single factor as the integration of these influences ultimately determines the pattern of behavior of a given bacterium.

Bacterial social interactions in mixed-species biofilms

Residing in a biofilm allows the bacteria to interact across species boundaries resulting in altered biofilm formation capabilities for food isolates. These interactions can be competitive, cooperative or neutral (Burmølle et al. 2014). The specific nature of the interactions will impact the biofilm and influence both the temporal and spatial properties of the formed biofilm. Fully grasping these interactions can be challenging but, if successful, will result in a better understanding of the driving interactions of a community and in turn the development of more efficient strategies for controlling unwanted biofilm formation.

Competitive interactions

Some of the most common competitive interactions are superior utilization of a given energy source (exploitative competition) or through the production of compounds that directly inhibit growth of other members (inference competition). Exploitative competition was recently demonstrated by Pang et al. (2019) who reported that indigenous microorganisms from fresh salmon runoff fluids, competing for nutrients in mixed-species biofilms with *L. monocytogenes*, were reduced in the number of cells compared to single-species biofilms. Furthermore, it has been demonstrated that competitive interactions are prevalent in mixed-species biofilms among phylogenetically and metabolically similar species, since the populations need to contend for nutrient sources and space (Russel et al. 2017).

Interference competition, i.e. the production of secondary metabolites or physiological by-products, such as bacteriocins, enzymes, hydrogen peroxide, and organic acids, also provides a competitive advantage over neighboring microorganisms within mixed-species biofilms (Rendueles and

Ghigo 2012). For *L. monocytogenes*, the biofilm formation was suppressed when co-cultured with *Lactobacillus paraplantarum*, mainly due to *Lactobacillus* metabolites such as hydrogen peroxide, lactic acid, and bacteriocins (Winkelströter, Tulini, and De Martinis 2015). Likewise, the competitive interaction in dual-species biofilm formation by *Shewanella baltica* and *Pseudomonas fluorescens* has been observed to result in lowered biomass and matrix polysaccharide production compared to single-species counterparts, which was associated with secreted compounds present in the culture supernatants of both spoilage strains (Zhu et al. 2019).

Another simple strategy for bacteria to gain a competitive advantage is avoiding the colonization of other species by rapid occupancy of all available adhesion sites, referred as 'surface blanketing'. For example, *Pseudomonas aeruginosa* rapidly spreads over the surface by swarming and twitching motility to prevent the adhesion of *Agrobacterium tumefaciens*, while the *P. aeruginosa flgK* motility-deficient knockout mutant, unable to spread quickly, was unable to exclude *A. tumefaciens* in a mixed-species biofilm (An et al. 2006).

Cooperative interactions

Bacterial interactions are categorized as cooperation when all the members in the community benefit in some way by the presence of the others, and this often leads to increased biofilm formation (Ren et al. 2014, Liu et al. 2016a). Studies demonstrating the relevance of cooperative interactions in food associated settings include examination of *Ralstonia insidiosa* and foodborne pathogens. *R. insidiosa* facilitates biofilm formation by *L. monocytogenes*, *S. enterica* and *E. coli* as *R. insidiosa* is highly efficient in nutrient utilization and proliferates well in oligonutrient environments, providing a micro-environment for bacterial accumulation and survival (Liu et al. 2016a). Røder et al. (2015) reported that approximately 20% of the multispecies consortia formed by isolates from a slaughterhouse showed enhanced biofilms formation 15 °C when comparing to mono-species biofilms.

Cooperative bacterial interactions may be based on metabolic cross-feeding facilitating the growth of other microorganisms. A cooperative dual-species community described by Kives et al. (2005) demonstrated that biofilms formed by *P. fluorescens* and *Lactococcus lactis* isolated from raw milk were structurally more complex and resulted in enhanced bacterial attachment by up to 100 and 20,000 folds for *P. fluorescens* and *L. lactis*, respectively. The poor biofilm former *L. lactis* benefitted from the enhanced attaching ability provided by the quickly developing matrix originating from *P. fluorescens* and in return, metabolites produced by *L. lactis* were utilized by *P. fluorescens* as nutrient sources.

Bacterial interactions in mixed-species biofilms are affected by many factors, as these depend on community members and environmental factors, thus affecting the structure, dynamics and properties of the biofilm community. To control pathogens in the food industry we need to understand how bacteria interact, and subsequently implement this in the design of optimal strategies for eradicating biofilms in food production and processing.

Resistance to disinfectants within mixed-species biofilms

Enhanced resistance to disinfectants within mixed-species biofilms

These biofilm communities assemble as a consequence of interspecies interactions, which not only dictate community structure and organization, but also functions. From the food industry perspective, one of the most alarming consequences of mixed-species biofilms is that they generally exhibit higher resistance to different disinfectants, such as benzalkonium chloride, sodium hypochlorite, peracetic acid and hydrogen peroxide when compared to mono-species biofilms (Table 2). For example, a significantly increased resistance to benzalkonium chloride was observed in dual-species biofilm formed by *L. monocytogenes* and *P. putida* after 4 days of incubation when compared to that of mono-species biofilms (Ibusquiza et al. 2012). Likewise, dual-species biofilms by *Serratia liquefaciens* and *Serratia putrefaciens* were more resistant to both ethanol and benzalkonium chloride than their mono-species counterparts (Liu et al. 2017).

Interestingly, the enhanced resistance to disinfectants may occur even when the overall productivity or fitness, measured as cell growth, of mixed-species biofilms decrease. For example, the presence of *P. aeruginosa* has been shown to reduce the cell density of *Salmonella* Enteritidis when mixed in dual-species biofilms, meanwhile protecting *Salmonella* cells from sanitizer treatment in a poultry processing environment (Pang and Yuk 2018). Thus, it is vital to examine the changes of each individual species in communities when the overall biomass, function or resistance of the community is enhanced, in order to determine whether cooperative or competitive interactions shape the system and how these interactions impact the tolerance towards antimicrobials.

Potential mechanisms of enhanced resistance to disinfectants within mixed-species biofilms

Unfortunately, the underpinning mechanisms of enhanced resistance to disinfectants within mixed-species biofilms still remain unclear. The changed composition of the matrix and enhanced EPS production in mixed-species biofilms have been hypothesized to be relevant for this, as the matrix varies greatly depending on the environmental conditions and bacterial species involved (Flemming and Wingender 2010). Accordingly, it has been suggested that chemical interactions between the polymers produced by different species may lead to a more viscous matrix, which provides a better defense against disinfectant treatments so that they hardly reach the deepest layers of the biofilm in their active form (Burmølle et al. 2006; Guillonneau et al. 2018).

Table 2. Examples of mixed-species biofilms showed increased resistance to disinfectants.

Microorganisms in mixed-species biofilms	Disinfections	Conditions for biofilm formation	References
Listeria monocytogenes and *Pseudomonas putida*	Benzalkonium chloride	Stainless steel and polypropylene coupons	Ibusquiza et al. (2012)
L. monocytogenes, *Pseudomonas fragi* and *Staphylococcus xylosus*	Sodium hypochlorite	The constant-depth film fermenter	Norwood and Gilmour (2000)
Bacillus cereus and *Pseudomonas fluorescens*	Glutaraldehyde	Stainless steel surfaces using a chemostat system	Simões et al. (2011)
B. cereus and *P. fluorescens*	Chlorine dioxide	Flow chamber	Lindsay et al. (2002)
Pseudomonas aeruginosa, *Salmonella* Typhimurium and *Salmonella* Enteritidis	Sodium hypochlorite and quaternary ammonium compounds	Stainless steel coupons	Pang, Yang, and Yuk (2017)
P. aeruginosa and *Salmonella* Enteritidis	Sodium hypochlorite	Stainless steel coupons	Pang and Yuk (2018)
Serratia liquefaciens and *Shewanella putrefaciens*	Benzalkonium chloride and ethanol	Stainless steel coupons	Liu et al. (2017)
Escherichia coli and *Pantoea agglomerans*	Amphoteric-based disinfectant	Stainless steel and other two DLC surface coatings	Gomes et al. (2018)
E. coli and *Salmonella enterica* Serovar Typhimurium	Quaternary ammonium chloride and chlorine solution	96-well polystyrene plates	Wang et al. (2013)
L. monocytogenes and *Lactobacillus plantarum*	Benzalkonium chloride and peracetic acid	12-well polystyrene microtiter plates	van der Veen and Abee (2011)
L. monocytogenes and *P. putida*	Benzalkonium chloride	Stainless steel coupons	Giaouris et al. (2013)
Bacillus subtilis and *Staphylococcus aureus*	Peracetic acid	Microtiter plates	Bridier et al. (2012)
L. monocytogenes and *P. aeruginosa*	Alkyl amine acetate based, chlorine based, and phosphoric acid based commercial dairy sanitizers	Calgary biofilm Device	Lourenço, Machado, and Brito (2011)
Burkholderia cepacia and *P. aeruginosa*	Chlorine dioxide	Silicone tubing	Behnke and Camper (2012)
B. cepacia and *P. aeruginosa*	Sodium hypochlorite	Silicone tubing	Behnke et al (2011)
E. coli O-:H4, *E. coli O157:H7*, *Salmonella* spp., *P. aeruginosa*, *Citrobacter* spp., *S. liquefaciens*, and *B. subtilis*	Hydrogen peroxide	Glass microscope slides	Uhlich, Rogers, and Mosier (2010)
S. enterica serovar Thompson or Newport with *P. fluorescens*	The washing aid T-128 mainly of phosphoric acid and propylene glycol	Stainless steel coupons	Shen et al. (2012)

A second explanation is that due to the specific spatial arrangement of certain bacterial species within a biofilm, some strains may be protected from a biocide by their aggregation with others within the differential three-dimensional structure (Lee et al. 2014). The organization of microorganisms within multispecies biofilms is not random, but follows a pattern that contributes to the fitness of the whole community (Liu et al. 2016b). Generally, there are three different types of bacterial organization patterns. Single-species microcolonies refer to an organization where each species forms a separate microcolony side by side (Figure 1A), which is a result of non-commensal interactions between species (Nielsen et al. 2000). Alternatively, biofilms can have a structure where species are mixed and identified together throughout the biofilm (Figure 1B), which is referred to intermixing (Rickard et al. 2006). Finally, biofilms can have a layered structure, where one species is located in the lower layers and the other species located in the upper layers (Figure 1C). This organization can enable both cooperative (Habimana et al. 2010), or competitive interactions (An et al. 2006). The spatial organizations partially determine the survival rate of the individual species when the biofilm is exposed to toxic compounds (Nadell, Drescher, and Foster 2016).

A third possible mechanism for enhanced tolerance in mixed-species biofilms has been speculated to involve transient changes in proximal neighbors, as one species residing within a mixed-species biofilm can significantly alter the physiology and thus enhance the resistance of neighboring species by interspecies interactions (Herschend et al. 2017; Hansen et al. 2017). Some interspecies interactions within mixed bacterial communities are governed by a cell-cell communication system known as quorum sensing (QS). QS relies on production and detection of autoinducers and orchestrates bacterial responses according to the level of these signal molecules. When a minimal threshold stimulatory concentration of autoinducers is reached, the QS system is activated and regulates gene expressions, including those regulating biofilm formation (Wang, Li, and Ling 2017). Another interaction which can have major consequences for the physiology of biofilms is the genetic exchange between biofilm residents. Biofilms facilitate horizontal gene transfer (HGT) due to high cell density and by promoting natural competence (Madsen et al. 2012). HGT is a key driver of bacterial diversification and enables dissemination of genetic material e.g. genes encoding antimicrobial resistance or other functionalities by conjugation, transduction or transformation, which can promote their persistence in food processing environments (Dubey and Ben-Yehuda 2011). Conjugative plasmids have been demonstrated to strongly induce biofilm formation within their hosts (Burmølle et al. 2014) and plasmids encoding resistance to several antibiotics have been shown to be readily transferred in dual-species biofilm of *P. putida* and *E. coli* (Meervenne et al. 2014).

The paradox of enhanced resistance to disinfectants within mixed-species biofilms

It is worth noticing that mixed-species biofilms are not always less susceptible towards disinfectants and

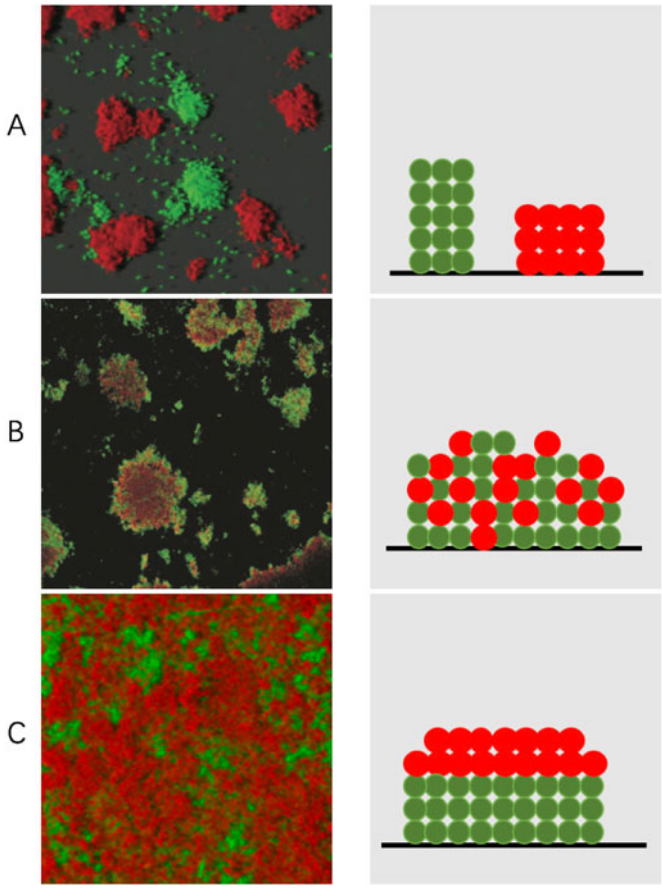

Figure 1. Spatial distribution within mixed-species biofilms: (A) separate mono-species microcolonies (Nielsen et al. 2000); (B) intermixing (Rickard et al. 2006); (C) arranged in layers (Habimana et al. 2010).

antimicrobials than biofilms composed of single-species, as the susceptibility to disinfectants can be attributed to many factors besides the number of species present. These include differences in food contact surfaces, types of disinfectants, experimental methods used, and the type of included micro-organisms and their specific interactions. For example, Kart et al. (2014) demonstrated that sensitivity towards anti-microbial agents depended on the identity rather than the number of species present within the mixed community as well as the specific disinfectant used. This phenomenon has also been reported in other studies (Iñiguez-Moreno et al. 2018; Lemos et al. 2015; Machado et al. 2012; Pang et al. 2019), indicating that increased tolerance towards disinfectants is not a universal trait for mixed-species biofilms.

Detection methods of mixed-species biofilms

Diverse technologies have been applied for revealing mixed-species biofilm structure, physiology, and interactions of different species on food contact surfaces (Table 3).

The most widely used technique to differentiate isolates from mixed-species biofilms is to dislodge sessile cells from food contact surfaces by swabbing, vortexing, or sonication, followed by a culture-based approach, and species dynamics in mixed biofilms can then be inferred from specific counts. However, critical sites, such as crevices and ends, where biofilms readily occur but are difficult to access, are challenging to sample (Winkelströter et al. 2014). *In situ* sensors have been developed to nondestructively monitor surfaces and evaluate the effectiveness of anti-biofilm treatment strategies in real-time. These include the optical deposit sensor based on multiple fluorescence excitation/emission matrix analysis (Strathmann et al. 2013) and a microfluidic sensor platform, based on the combination of electrical impedance spectroscopy and amperometric current measurement designed for online measurement of biofilm formation and activity (Bruchmann et al. 2015).

Some bacteria in biofilms are subjected to various stresses in food processing environments such as starvation, chemicals, and heat that may injure cells and render them non-culturable, but do not necessarily imply a complete inactivation or absence of bacterial cells (Ramamurthy et al. 2014). To overcome this, high-throughput sequencing and next-generation sequencing technologies could provide more sensitive alternatives to identify the inhabitants of mixed-species biofilms present in food production facilities (Valera et al. 2015; Dixon et al. 2018; Rice, Wuertz, and Kjelleberg 2016).

Once bacterial isolates have been isolated from the food production facility, they can be characterized by various methods. These, however, all depend on the successful isolation and cultivation of pure bacterial cultures and are thus limited to examination of the culturable fraction of the bacteria present at the relevant site. Crystal Violet (CV) staining is a high-throughput method to quantify the ability to attach and form biofilm by a large number of bacterial species under different conditions (Yuan et al. 2018a). Absence of a standardized protocol might, however, be a drawback of this technique and makes comparison of results between different studies difficult and leads to poor reproducibility. Also, the nonspecific nature of CV does not allow species differentiation in polymicrobial communities.

Molecular technologies such as quantitative polymerase chain reaction (qPCR), DNA microarray, and the field of omics approaches are instrumental in the identification of gene regulation and virulence of mixed-species biofilms in specific food niches. qPCR is a molecular tool for the identification and quantification of specific microorganisms and their genes in mixed-species communities (Machado, Jefferson, and Cerca 2013). This technique allows discrimination between live and dead cells when combined with amplification of rRNA regions and propidium monoazide (PMA), as PMA can penetrate compromised or damaged membranes, intercalate DNA, and prevent its amplification (Yasunaga et al. 2013). However, viable cells with only a slightly damaged cell membrane might not be accounted for, and the presence of a high number of dead cells could affect viable cell quantification (Azeredo et al. 2017). Alternatively, DNA microarrays have successfully been employed to demonstrate significant differences in gene expression in mixed-species biofilms (Pammi et al. 2013). To study global changes and variations at gene-, RNA-, protein- and metabolic levels, different omics approaches, namely genomics, proteomics, transcriptomics and metabolomics, have been successfully used to reveal the mechanisms driving community development and structure. These techniques provide

Table 3. Methods for studying the composition, structure and bacterial interactions of mixed-species biofilms.

Methods	Application	Advantages	Disadvantages
Dislodge sessile cells from food contact surfaces followed by plate counting	Assesses the composition and bacterial interactions in mixed-species biofilms	Easy to conduct	Time-consuming; hard to access to critical zones; non-culturable cells cannot be detected; underestimation of cells that present at a negligible level in mixed-species biofilms
Crystal violet staining	Screening for the total biofilm biomass	Cheap, high-throughput, and easy to conduct	No standardized protocol, and results are laboratory-dependent
Quantitative polymerase chain reaction (qPCR)	Assesses the total number of cells	Quick, and enables quantification of different species within one sample	Expensive; overestimates the number of cells due to the presence of eDNA
qPCR combined with propidium monoazide (PMA)	Assesses total number of cells by PCR with a prior treatment of the sample with PMA that inactivates eDNA and DNA of dead cells	Quick, and able to quantify only viable cells	Assesses viability only based on membrane cell integrity
Omics	Provide information on how participating members cooperate and compete, and how metabolic activities are distributed between community members at gene-, RNA-, protein- and metabolic levels	Large-scale acquisition of biofilm characteristics	Analyzing the sequence information is complicated and time-consuming; provide only the average result for an entire; any unique pattern for an underrepresented subpopulation can be overlooked
Scanning electron microscopy	Study of the biofilm spatial structure	Resolution higher than other imaging techniques; ability to image complex shapes	Time-consuming preparation procedures; the biofilm structure may be altered when dehydrating biofilm samples during preparation
Confocal laser scanning microscopy	Differentiate between mixed-species biofilms; biofilm visualization and quantification of structural parameters; spatial distribution of viable bacteria, localized cell death	Non-destructive investigation of biofilm structures with high resolution; the elimination of defocused haze; quantitative visualization of 3-D and 4-D biofilms	Low-magnification spatial images of how bacteria are located and interact within the biofilm; interference of local properties of the biofilm with the fluorescence probes
Fluorescence *in situ* hybridization	Analyze the composition and localization of microbial species biofilms	Fast and accurate detection of specific DNA; can be performed even in non-actively dividing cells	Relatively low-throughput; low probe permeability; poor probe hybridization efficiency; very limited number of different; target organisms that can be detected simultaneously
Atomic force microscopy	Determination of adhesion forces between biofilm and substratum, as well as cohesive strength	Nondestructive technique; minimizing pretreatment procedures and occurrence of artifacts; provide 3-D images of the surface topography; quantitative assessment of biofilm interaction with surfaces and biofilm cohesion	Inability to obtain a large area survey scan and to image side-walls of bacterial cells; damaging of the soft and gelatinous nature of biofilms by the imaging of the surface; the need for immobilization of cells during imaging

information on how participating members cooperate and compete for resources and how metabolic activities are distributed between community members (Verastegui et al. 2014; Nakamura et al. 2016; Herschend et al. 2017).

Microscopy techniques, such as scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), fluorescence *in situ* hybridization (FISH), continuous-optimizing confocal reflection microscopy (COCRM), laser ablation electrospray ionization mass spectrometry (LAESI-MS) and atomic force microscopy (AFM), provide a better understanding of the complex structure of biofilms, its relationship with surface substratum, microbial morphology, and species composition. SEM has higher resolution and may show the location of each bacterium and three-dimensional structure of biofilms; however, as biofilms mainly consist of water, the biofilm structure may be altered when dehydrating biofilm samples during preparation (Alhede et al. 2012). CLSM is the tool used to differentiate between different species in mixed biofilms when coupled with fluorescent protein markers (Habimana et al. 2010) or fluorescent *in situ* hybridization (FISH) (Liu et al. 2018), because it enables the direct investigation of biofilm structures with high resolution, the elimination of defocused haze, and quantitative visualization of two-dimensional, three-dimensional and four-dimensional reconstructions of biofilm. The main disadvantage of CLSM is the low-magnification spatial images of how bacteria are located and interact within the biofilm (Alhede et al. 2012). More importantly, CLSM imaging with live/dead staining should be carefully analyzed since differentiation between the red or green channels is often biased by the intensity of the lasers (Azeredo et al. 2017). FISH represents an accurate but relatively low-throughput quantitative analysis of the composition and localization of microbial species in biofilms. Many advanced versions of FISH have been implemented for different purposes: peptide nucleic acid-FISH (PNA-FISH) for better probe penetration through the biofilm matrix and the cellular envelope, catalyzed reporter deposition-FISH (CARD-FISH) for fluorescent signal enhancement and combinatorial labeling and spectral imaging FISH (CLASI-FISH) for expanding the number of distinguishable taxa in complex communities (Costa et al. 2017). COCRM is a useful method for visualizing the process of the development of mixed-species biofilm formation, and can be used under conditions that are unsuitable for fluorescent protein, such as high or low temperature, low pH or anaerobic conditions since this

procedure does not necessitate a staining procedure (Inaba et al. 2013). Fast, direct, noninvasive LAESI-MS analysis of mixed-species biofilms has profound implications for the study of biofilms, as this innovative technique eliminates the need for lengthy and disruptive sample preparation and provides previously unattainable information on both the structure of biofilms and the chemical effects of anti-biofilm treatment (Dean et al. 2015). Finally, AFM is a powerful technique to gain noninvasive imaging of critical dimensions such as cell size, appendage length and surface roughness. The limitations are, however, the inability to obtain a large area survey scan and observe the side walls of bacterial cells, and the soft and gelatinous nature of the biofilm (Peck, Chew, and Bird 2019).

In recent years, the development of mathematical modeling tools focused on bacterial interactions within biofilms has emerged, and such models contribute to developing a comprehensive understanding of complex microbial ecosystems in food processing environments. For example, Zhao et al. (2016) developed a 3D multiphasic hydrodynamic model and numerical tool for fluid-structure biofilms to study mechanisms of antimicrobial persistence in heterogeneous multi-species biofilms, and identified young biofilms as more susceptible to anti-microbial treatment than mature ones.

Novel strategies to control and eradicate mixed-species biofilms

Since mixed-species biofilms are a great concern in relation to food safety, extensive studies have been conducted to minimize the accumulation of biofilms in food industry settings or to develop novel removal methods that are more effective, economical and sustainable. Daily cleaning and disinfection processes are carried out in every food manufacturing plant to prevent bacterial colonization or persistence on food contact surfaces. Antimicrobial chemicals, including chlorine, hydrogen peroxide, and peracetic acid, have been extensively used for many years. However, such approaches are not always effective for biofilm control as residual microorganisms on equipment surfaces can still be found after cleaning and sanitizing procedures, particularly with respect to the removal of recalcitrant bacterial biofilms formed on surfaces (Fagerlund et al. 2017; Parijs and Steenackers 2018; Maes et al. 2019). Other drawbacks of current cleaning and disinfection regimes are the corrosion of equipment, and the possible toxicity to users and environment (Bridier et al. 2011). More seriously, the continuous and inappropriate use of disinfectants may favor biofilm formation and tolerance instead of eradicating biofilms, by selecting for tolerant and resistant biofilm forming strains (Rodríguez-Lopez and Cabo 2017; Machado et al. 2012). It should also be noted that although EPS and the biofilm structure are considered determining factors in biofilm resistance, the ability of an antimicrobial agent to penetrate a biofilm is not correlated with its killing or removal efficiency (Araújo et al. 2014). Therefore, for biofilm eradication, a thorough optimization, not only of the right amounts of antimicrobial compounds utilized but also a proper time scheduling, is necessary prior to the application of sanitation procedures in order to obtain proper bactericidal effects while avoiding selection for resistant variants.

As conventional control methods sometimes fail to remove adhered bacteria from the process equipment, new alternative strategies are urgently needed for efficient and continuous prevention of mixed-species biofilms (Figure 2).

Bacteriophages

One alternative to inefficient conventional treatment strategies is the therapeutical use of bacteriophages (phages). Phages are viruses of bacteria and phage therapy provide a natural, highly specific and nontoxic approach to combat biofilms. When phages encounter biofilms, the outcome depends on the susceptibility of the matrix-embedded bacteria and the availability of receptor sites, potentially hidden by a physical barrier i.e. biofilm matrix (Sutherland et al. 2004; Gutiérrez et al. 2016). Phages are however highly diverse and some encode depolymerases enabling them to degenerate and penetrate the biofilm matrix and subsequently lyse bacteria (Endersen et al. 2014). The applicability of such enzymes in biofilm eradication was evident when Lu and Collins (2007) managed to engineer the coliphage T7 to encode and express the depolymerase dispersin B during infection. The constructed phage enabled significantly higher disruption of biofilm formation than the wild-type T7 phage. Another great example of genetic phage engineering in order to improve their therapeutical potential was presented by Pei and Lamas-Samanamud (2014). They reported that an engineered T7 bacteriophage encoding the AiiA lactonase enzyme with broad-range activity for quenching of QS, inhibited the mixed-species biofilm formation of *P. aeruginosa* and *E. coli*.

Although the high specificity of phages is an advantage in the context of maintaining the commensal microbiota, it might also prove to be a drawback, as the limited host range require identification of the target bacteria prior to treatment. The use of phage cocktails, engineered phages, combination of phages with other antimicrobials, in addition to the application of purified phage enzymes e.g. endolysins could provide effective strategies to overcome this hurdle (Bárdy et al. 2016).

The infection of biofilm cells by phages is extremely conditioned by their chemical composition and environmental factors, such as temperature, growth stage, media and phage concentration. Encapsulation technologies have been successfully used to increase phage stability in the extreme environmental conditions encountered in many food-processing facilities, preserve their activity and enable their release in targeted environments (Hussain et al. 2017). Phage diffusion in biofilms is a fundamental factor for the outcome of phage-bacteria encounters (Simmons et al. 2018) and production of the amyloid fiber, curli, plays an essential role in this context (Vidakovic et al. 2018). Thus, future research of phage therapy needs to elucidate the temporal development of matrix components and how mixed-species

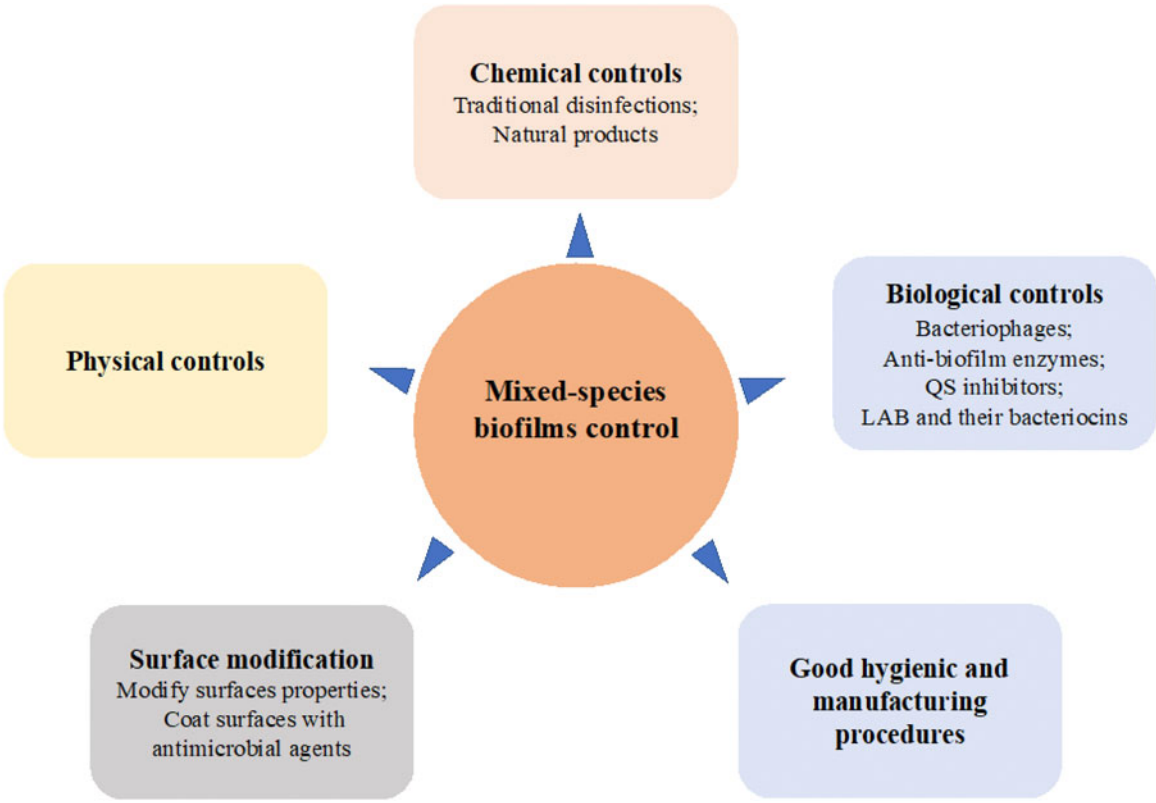

Figure 2. A summary of mixed-species biofilm control strategies.

biofilm affects this composition in relation to phage sensitivity. A regulatory framework for phage applications should also be established to boost investment in phage-based products.

Anti-biofilm enzymes

The EPS matrix provides advantages to bacterial cells embedded in biofilms enhancing their persistence on food contact surfaces. The use of enzymes as 'green chemicals', or combined with biocides, can serve as an alternative biofilm removal approach due to their capability of degrading the EPS network, facilitate penetration of cleaning and disinfection agents, interrupt the cell-to-cell signaling events governing biofilm formation and maintenance, and would also help to reduce the use of chemical agents, water consumption, and energy cost (Nahar et al. 2018; Rodríguez-López et al. 2017).

In general, there are four types of enzymes of particular interest for biofilm removal: anti-QS enzymes, oxidative enzymes, polysaccharide-degrading enzymes and proteolytic enzymes (Meireles et al. 2016). Anti-QS enzymes interrupt the cell-to-cell signaling events governing biofilm formation and maintenance. Oxidative enzymes can target eDNA present in the biofilm matrix, and either prevent biofilm formation and maturation, cause dispersal, or facilitate antimicrobial exposure of biofilms cells. Polysaccharide-degrading enzymes include amylase, alginate lyase, cellulase and lysozyme. Proteases, a diverse group of enzymes that differ in structure, target substrate, and reaction mechanism, have been used to hydrolyze proteins present in the matrix, or related proteins which enable bacteria to attach to a surface.

However, the EPS composition in biofilms varies greatly from one bacterial species to another and is strongly affected by growth conditions (Flemming and Wingender 2010). Taking into account the substrate-specificity of enzymes combined with the complex heterogeneous composition of the biofilm matrix, the successful removal of biofilms usually requires a combination of different enzymes capable of degrading DNA, polysaccharides, proteins and QS molecules and increasing the range of action of an enzymatic cleaner against mixed-species biofilms (Nahar et al. 2018; Lequette et al. 2010).

The use of enzymes in biofilm control is currently limited due to high cost and low commercial accessibility of different enzymes, as many environmental conditions in the food industry, such as temperature, pH, substrate, food residues, and varieties of food processing surfaces, are the key factors that may interfere with the activity and efficiency of enzymes and there challenge the treatment procedure (Nahar et al. 2018).

Natural products

The discovery of effective anti-biofilm compounds originating from plants, such as essential oils, curcumin, phenolic acids, polyphenolic compounds, has gained much interest from researchers over the years.

Essential oils are natural compounds, which potential application as alternative natural disinfectants suitable for control of mixed-species biofilm. Kerekes et al. (2013)

reported a 2-log reduction of cell numbers in mixed-species biofilms treated with lemon based essential oils. Likewise, dual-species biofilm by *S. aureus* and *S. enterica* Serovar Typhimurium formed under the influence of continuously low carvacrol concentrations failed to mature into a steady-state biofilm, and did not reach any stage of biofilm formation in the presence of high concentrations (Knowles et al. 2005), as carvacrol disturbs bacterial membranes leading to leakage of intracellular ATP and potassium ions, and ultimately cell death (Ultee, Kets, and Smid 1999).

It should be noted that the practical application of essential oils for disinfection of microbial contaminated industrial surfaces may be hampered by the strong smell and the subsequent difficulties of efficient flushing from surfaces after a disinfection program. Interestingly, Chorianopoulos et al. (2008) showed the antimicrobial action of the hydrosol fraction of *Satureja thymbra* essential oils against mixed-species biofilms composed of spoilage and pathogenic meat bacteria. This hydrosol fraction is an aqueous solution which is easily rinsed from surfaces, and does not have the strong smell.

Other natural plant extracts have also been reported to have the capacity to control biofilms. Other natural plant extracts have also been reported to have the capacity to control biofilms. For example, in the presence of phenolic acids (ferulic and salicylic acids), dual-species biofilm by *Bacillus cereus* and *P. fluorescens* were highly susceptible to a secondary exposure to these chemicals. It was demonstrated that the continuous exposure of dual-species biofilms to phenolic acids decreased their resilience and resistance to inactivation and removal, probably due to an interference with the interactions shaping the dual-species biofilm formation (Lemos et al. 2014). In addition, rutin was shown to significantly inhibit the multi-species biofilm formation by foodborne drug resistant *E. coli* and *S. aureus* at sub-lethal concentrations (Al-Shabib et al. 2017). Cuminaldehyde and indole-3-carbinol were proved as green and sustainable sources of antimicrobial potentiators against *E. coli* and *S. epidermidis* dual-species biofilm at concentrations close to the minimum bactericidal concentration when combined with EDTA (Vale et al. 2019).

Indeed, these findings indicate that different plant extracts have inhibitory effects on biofilms of many organisms. Further research should study the underlying mechanisms in detail in order to elucidate the true potential of these as anti-biofilm agents.

Lactic acid bacteria (LAB) and their bacteriocins

Application of bacteriocins or their producer strains for the inhibition of mixed-species biofilm formation is a novel field of research (Hossain, Sadekuzzaman, and Ha 2017). Interestingly, the co-culture of *Lactococcus lactis* subsp. *lactis* with *L. monocytogenes* on stainless steel resulted in a 4-log reduction of *L. monocytogenes* cell numbers (Dygico et al. 2019). Likewise, the numbers of *L. monocytogenes* biofilm cells during the competition, exclusion and displacement assays were effectively reduced by more than 3-log when co-cultured with *L. paracasei* and *Lactobacillus rhamnosus* (Woo and Ahn 2013).

Bacteriocins of LAB, recognized as 'generally regarded as safe' (GRAS) and thus applicable for food production, including application as food additives, have been used to impede initial cell adhesion and biofilm formation. For example, cell-free supernatants of *Lactobacillus gasseri* and *L. rhamnosus* exerted an anti-biofilm effect on mixed non-albicans *Candida* species biofilm by reducing the biomass as well as inhibiting the metabolic activity, and disrupting the pre-formed biofilm when added during the biofilm formation process (Tan et al. 2018).

New surfaces for biofilm prevention

The properties of food contact surfaces determine biofilm onset and resistance in food processing environments. Thus, the modification of surfaces either by altering physico-chemical properties, such as reduction of free energy or coating of surfaces are considered as the emerging approaches to prevent biofilm formation in food industry (Gule, Begum, and Klumperman 2016).

Diamond-like carbon coatings, approved as food contact surfaces, have been examined as alternatives to non-coated stainless steel in food plants due to their high hardness, low friction, chemical inertness, high wear-resistance and anti-fouling properties. The higher efficiency by diamond-like carbon coatings in inactivation of *E. coli* indicates that these surfaces may be used in critical sites of food processing plants where bacterial attachment is more likely to occur and disinfection is difficult (Gomes et al. 2018). Lower mesophilic and thermophilic bacterial counts, and less organic matter were observed on the surface of Ni-P-polytetrafluoroethylene (PTFE)-coated stainless steel plate heat exchangers during a 17-h milk pasteurization session (Jindal et al. 2018). Suitable organo-silanes can be used to improve the physical, chemical and mechanical properties of materials, which may prevent biofilms formation in the food industry (Kregiel 2014).

The use of antimicrobial agents to control biofilms in industrial settings is increasing, although the majority of research is still in the developmental stage. The adsorption of biosurfactants to a solid surface can modify its hydrophobicity affecting the adhesion process and consequently the biofilm formation. For example, conditioning of polystyrene with surfactin from *B. subtilis* and rhamnolipids from *P. aeruginosa* reduced the hydrophobicity of the surface, delayed the adhesion of food pathogenic bacteria in a nutrient rich environment, and disrupted mixed-species biofilm formation by *L. monocytogenes*, *S. aureus* and *Salmonella* Enteritidis (Gomes and Nitschke 2012). Treatment of linear low-density polyethylene with 10% of the antimicrobial poly((tert-butylamino)-methyl-styrene) can reduce and delay the formation of mixed-species biofilms of the pathogens *S. aureus*, *L. monocytogenes* and *E. coli* (Hüwe et al. 2018).

QS inhibitors (QSIs)

Disabling QS circuits and hence block coordinated behavior of communities is a particularly interesting strategy.

Although much still needs to be investigated in order to fulfill the potential of this strategy, QSIs have been evaluated as promising anti-biofilm agents. Strategies employed to prevent biofilm formation targeting the QS system are based on inhibition of cell-to-cell communication, which can be executed in a number of ways, including the inhibition of autoinducers synthesis or the degradation of autoinducers, prevention of signaling peptide-receptor binding or inhibition of the signal transduction cascade pathways (Yuan et al. 2018b). For example, vanillin has been found to be a promising QSI, reducing the biomass of mixed-species biofilms by 9%, 25% and 52% post 24 hours of incubation in the presence of 0.05, 0.15 and 0.30 mg/mL vanillin, respectively. Further examination of the effect found that vanillin significantly reduced production of exopolysaccharides (17%) and exoproteins (28%) (Si and Quan 2017). Similarly, the antimicrobial peptide, helical cathelicidin peptide LL-37, dysregulated QS in *P. aeruginosa*, and enabled inhibition of dual-species biofilm formation by *S. aureus* and *P. aeruginosa* (Dean et al. 2015). However, QSIs have the disadvantages of being rather specific and thus a mixture of QSIs may be needed to inhibit biofilm formation of a mixed community.

Physical treatment

Novel physical microbial inactivation technologies are also proposed as alternative green technologies for biofilm control in food industries. Non-thermal atmospheric plasmas have received much attention, as they have demonstrated high disinfectant capacity against biofilms of a broad spectrum of microorganisms and offer a series of advantages over conventional chemical disinfection, such as rapid, being contact-free and waterless (Pignata et al. 2017). Bacterial populations in mixed-species biofilms treated with high voltage (80 kV) atmospheric cold plasma for 60 s were significantly reduced in cell numbers, resulting in non-detectable levels after extending treatment time to 120 s (Patange et al. 2019). However, the impact on the organoleptic and nutritional characteristics of foods needs to be taken into consideration in the future. Other physical surface decontamination technologies that have been developed or investigated in recent years for the inactivation of microorganisms within biofilms are steam heating (Ban and Kang 2016), ozone treatment (Varga and Szigeti 2016), and Ultraviolet C irradiation (Jahid et al. 2014).

Hurdle technology

Although no single approach can currently address the problem of undesirable biofilms, the combination of two or more control techniques, called 'hurdle approach', could more effectively remove biofilm organisms from food processing facilities, as they would attack the microorganisms in different ways followed by an overall effective reduction in bacterial contamination.

Ban and Kang (2016) reported that the combination treatment of sanitizers with steam produces a synergistic effect by reducing mixed-species biofilm formation by *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* as evidenced by plating counts and imaging. The most effective combination for reducing numbers of pathogen biofilm cells was the combined treatment of steam and iodophor; steam for 20 s and merely 20 ppm iodophor for 30 s, which resulted in cell numbers below the detection limit. The combination of levulinic acid and sodium dodecyl sulfate showed a synergistic antimicrobial effect on inactivating bacterial cells in mixed-species biofilms by *E. coli* O157:H7 and *Salmonella*, as the combination of this organic acid and surfactant may promote the detachment of bacterial cells from a biofilm matrix and kill the detached cells by the undissociated levulinic acid (Chen, Zhao, and Doyle 2015). Vanillin in combination with proteinase K or DNase I was more effective in reducing biomass attachment than the treatment with the inhibitors in isolation, suggesting a synergetic function between vanillin and proteinase K or DNase I. This was possibly due to the spider-web like nature of the biofilm EPS matrix that trapped vanillin and lowered its penetration rate into the biofilm. The presence of proteinase K or DNase I may reduce the amount of EPS associated with suspended bacteria and initially attached bacteria, which in turn increases the availability of vanillin, thereby enhancing its efficiency as a biofilm inhibitor (Si and Quan 2017). Likewise, Rodríguez-López et al. (2017) stated that a combination of pronase and benzalkonium chloride effectively removed the late-stage dual-species biofilm by *L. monocytogenes* and *E. coli*.

The above-mentioned strategies have all shown promising application potential under laboratory settings, but they might meet limitations when upscaled and applied in the food industry. Firstly, anti-biofilm agents should be proven safe for application in the food industry. Additionally, quality of food products is a top priority for food manufacturers, and biofilm inhibitors must not influence the taste and texture of food products. Another important factor to consider is the practicality of the biofilm-fighting strategy proposed in an industrial setting, as they must be capable of withstanding harsh conditions in food processing environment (Coughlan et al. 2016).

From a practical viewpoint, at present, the most cost-effective option is to control mixed-species biofilms by strict adherence to hygiene standards. Improving pre-requisite programs through facility maintenance, equipment design, increased hygiene awareness of farmers, and the implementation of sanitation standard operating procedures (SSOP) or good manufacturing practices (GMP), is obviously important for minimizing cross-contamination, preventing biofilms formation and improving the safety and quality of foods (Lelieveld, Holah, and Napper 2013).

Concluding remarks

Mixed-species biofilms are frequently found in food industry if sanitation procedures are inadequate, and there is a growing concern regarding mixed-species biofilms in food production facilities. It is clear that bacterial interactions across

species boundaries enable functions not possible in monocultures or predictable from studying these. While the understanding of interactions in mixed-species biofilms is still very limited, studies are beginning to unravel the complexity and importance of interspecies interactions and their impact in food industry. This is of great importance as the majority of biofilms in the food industry are mixed-species and sanitation procedures are often inadequate. Further knowledge regarding mixed-species communities, their role in biofilm persistence, food safety and quality, and cutting-edge detection methods are required in the emerging field of food microbiology.

Novel strategies of biofilm eradication, such as the use of bacteriophages, enzymes, essential oils, surfaces modifications, and QS inhibitors, represent promising alternatives to control mixed-species biofilms. In the future, it is necessary to focus more closely on associations with particular food niches using omics, as well as novel and green technology to combat mixed-species biofilms. The field, however, is still awaiting innovative tools that will facilitate molecular and biochemical characterization on a single cell level within the context of mixed communities that will allow us to finally decipher exactly who is doing what and when. These new approaches may enhance the understanding of the molecular basis of biofilm regulatory pathways and will advance the development of new strategies and technologies to control microorganisms in biofilms.

Funding

This work was supported by the National Natural Science Foundation of China (31772080) and China Scholarship Council.

ORCID

Lei Yuan http://orcid.org/0000-0002-3139-7800
Mette Burmølle http://orcid.org/0000-0003-1870-632X

References

Acker, H. V., P. V. Dijck, and T. Coenye. 2014. Molecular mechanisms of antimicrobial tolerance and resistance in bacterial and fungal biofilms. *Trends in Microbiology* 22 (6):326–33. doi: 10.1016/j.tim.2014.02.001.

Alavi, H. E. D., and L. T. Hansen. 2013. Kinetics of biofilm formation and desiccation survival of *Listeria monocytogenes* in single and dual species biofilms with *Pseudomonas fluorescens*, *Serratia proteamaculans* or *Shewanella baltica* on food-grade stainless steel surfaces. *Biofouling* 29 (10):1253–68. doi: 10.1080/08927014.2013.835805.

Alhede, M., K. Qvortrup, R. Liebrechts, N. Høiby, M. Givskov, and T. Bjarnsholt. 2012. Combination of microscopic techniques reveals a comprehensive visual impression of biofilm structure and composition. *FEMS Immunology & Medical Microbiology* 65 (2):335–42. doi: 10.1111/j.1574-695X.2012.00956.x.

Al-Shabib, N. A., F. M. Husain, I. Ahmad, M. S. Khan, R. A. Khan, and J. M. Khan. 2017. Rutin inhibits Mono and multi-species biofilm formation by foodborne drug resistant *Escherichia coli* and *Staphylococcus aureus*. *Food Control* 79:325–32. doi: 10.1016/j.foodcont.2017.03.004.

Alvarez-Ordóñez, A., L. M. Coughlan, R. Briandet, and P. D. Cotter. 2019. Biofilms in food processing environments: Challenges and opportunities. *Annual Review of Food Science and Technology* 10 (1):173–95. doi: 10.1146/annurev-food-032818-121805.

An, D., T. Danhorn, C. Fuqua, and M. R. Parsek. 2006. Quorum sensing and motility mediate interactions between *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens* in biofilm cocultures. *Proceedings of the National Academy of Sciences of the United States of America* 103 (10):3828–33. doi: 10.1073/pnas.0511323103.

Anand, S., A. Hassan, and M. Avadhanula. 2012. The effects of biofilms formed on whey reverse osmosis membranes on the microbial quality of the concentrated product. *International Journal of Dairy Technology* 65 (3):451–5. doi: 10.1111/j.1471-0307.2012.00848.x.

Araújo, E. A., N. J. de Andrade, L. H. M. da Silva, A. F. de Carvalho, C. A. de Sá Silva, and A. M. Ramos. 2010. Control of microbial adhesion as a strategy for food and bioprocess technology. *Food and Bioprocess Technology* 3 (3):321–32. doi: 10.1007/s11947-009-0290-z.

Araújo, P. A., F. Mergulhão, L. Melo, and M. Simões. 2014. The ability of an antimicrobial agent to penetrate a biofilm is not correlated with its killing or removal efficiency. *Biofouling* 30 (6):675–83. doi: 10.1080/08927014.2014.904294..

Azeredo, J., N. F. Azevedo, R. Briandet, N. Cerca, T. Coenye, A. R. Costa, M. Desvaux, G. Di Bonaventura, M. Hébraud, Z. Jaglic, et al. 2017. Critical review on biofilm methods. *Critical Reviews in Microbiology* 43 (3):313–51. doi: 10.1080/1040841X.2016.1208146.

Ban, G. H., and D. H. Kang. 2016. Effect of sanitizer combined with steam heating on the inactivation of foodborne pathogens in a biofilm on stainless steel. *Food Microbiology* 55:47–54. doi: 10.1016/j.fm.2015.11.003.

Bárdy, P., R. Pantůček, M. Benešík, and J. Doškař. 2016. Genetically modified bacteriophages in applied microbiology. *Journal of Applied Microbiology* 121 (3):618–33. doi: 10.1111/jam.13207.

Behnke, S., and A. K. Camper. 2012. Chlorine dioxide disinfection of single and dual species biofilms, detached biofilm and planktonic cells. *Biofouling* 28 (6):635–47. doi: 10.1080/08927014.2012.700705.

Behnke, S., A. E. Parker, D. Woodall, and A. K. Camper. 2011. Comparing the chlorine disinfection of detached biofilm clusters with those of sessile biofilms and planktonic cells in single- and dual-species cultures. *Applied and Environmental Microbiology* 77 (20):7176–84. doi: 10.1128/AEM.05514-11.

Bridier, A., R. Briandet, V. Thomas, and F. Dubois-Brissonnet. 2011. Resistance of bacterial biofilms to disinfectants: A review. *Biofouling* 27 (9):1017–32. doi: 10.1080/08927014.2011.626899.

Bridier, A., M. D. P. Sanchez-Vizuete, D. L. Coq, S. Aymerich, T. Meylheuc, J. Y. Maillard, V. Thomas, F. Dubois-Brissonnet, and R. Briandet. 2012. Biofilms of a *Bacillus subtilis* hospital isolate protect *Staphylococcus aureus* from biocide action. *PLoS One* 7 (9):e44506. doi: 10.1371/journal.pone.0044506.

Brooks, J. D., and S. H. Flint. 2008. Biofilms in the food industry: Problems and potential solutions. *International Journal of Food Science & Technology* 43 (12):2163–76. doi: 10.1111/j.1365-2621.2008.01839.x.

Bruchmann, J., K. Sachsenheimer, B. E. Rapp, and T. Schwartz. 2015. Multi-channel microfluidic biosensor platform applied for online monitoring and screening of biofilm formation and activity. *PLoS One* 10 (2):e0117300. doi: 10.1371/journal.pone.0117300.

Buchanan, R. L., L. G. M. Gorris, M. M. Hayman, T. C. Jackson, and R. C. Whiting. 2017. A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control* 75:1–13. doi: 10.1016/j.foodcont.2016.12.016.

Burmølle, M., D. Ren, T. Bjarnsholt, and S. J. Sørensen. 2014. Interactions in multispecies biofilms: Do they actually matter? *Trends in Microbiology* 22 (2):84–91. doi: 10.1016/j.tim.2013.12.004.

Burmølle, M., J. S. Webb, D. Rao, L. H. Hansen, S. J. Sørensen, and S. Kjelleberg. 2006. Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. *Applied and Environmental Microbiology* 72 (6):3916–23. doi: 10.1128/AEM.03022-05.

Chamberland, J., M. H. Lessard, A. Doyen, S. Labrie, and Y. Pouliot. 2017. A sequencing approach targeting the 16S rRNA gene unravels the biofilm composition of spiral-wound membranes used in the

dairy industry. *Dairy Science & Technology* 96 (6):827–43. doi: 10.1007/s13594-016-0305-2.

Chamberland, J., T. Messier, E. Dugat-Bony, M. H. Lessard, S. Labrie, A. Doyen, and Y. Pouliot. 2019. Influence of feed temperature to biofouling of ultrafiltration membrane during skim milk processing. *International Dairy Journal* 93:99–105. doi: 10.1016/j.idairyj.2019.02.005.

Chen, D., T. Zhao, and M. P. Doyle. 2015. Single- and mixed-species biofilm formation by *Escherichia coli* O157:H7 and *Salmonella*, and their sensitivity to levulinic acid plus sodium dodecyl sulfate. *Food Control* 57:48–53. doi: 10.1016/j.foodcont.2015.04.006.

Cherif-Antar, A., B. Moussa-Boudjemâa, N. Didouh, K. Medjahdi, B. Mayo, and A. B. Flórez. 2016. Diversity and biofilm-forming capability of bacteria recovered from stainless steel pipes of a milk-processing dairy plant. *Dairy Science & Technology* 96 (1):27–38. doi: 10.1007/s13594-015-0235-4.

Chorianopoulos, N. G., E. D. Giaouris, P. N. Skandamis, S. A. Haroutounian, and G. J. E. Nychas. 2008. Disinfectant test against monoculture and mixed-culture biofilms composed of technological, spoilage and pathogenic bacteria: Bactericidal effect of essential oil and hydrosol of *satureja thymbra* and comparison with standard acid-base sanitizers. *Journal of Applied Microbiology* 104 (6): 1586–96. doi: 10.1111/j.1365-2672.2007.03694.x.

Cleto, S., S. Matos, L. Kluskens, and M. J. Vieira. 2012. Characterization of contaminants from a sanitized milk processing plant. *PLoS One* 7 (6):e40189. doi: 10.1371/journal.pone.0040189.

Costa, A. M., F. J. Mergulhão, R. Briandet, and N. F. Azevedo. 2017. It is all about location: How to pinpoint microorganisms and their functions in multispecies biofilms. *Future Microbiology* 12 (11): 987–99. doi: 10.2217/fmb-2017-0053.

Coughlan, L. M., P. D. Cotter, C. Hill, and A. Alvarez-Ordóñez, 2016. New weapons to fight old enemies: Novel strategies for the (bio)-control of bacterial biofilms in the food industry. *Frontiers in Microbiology* 7:1641. doi: 10.3389/fmicb.2016.01641.

Dean, S. N., C. Walsh, H. Goodman, and M. L. van Hoek. 2015. Analysis of mixed biofilm (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) by laser ablation electrospray ionization mass spectrometry. *Biofouling* 31 (2):151–61. doi: 10.1080/08927014.2015.1011067.

Dhowlaghar, N., M. Bansal, M. W. Schilling, and R. Nannapaneni. 2018. Scanning electron microscopy of *Salmonella* biofilms on various food-contact surfaces in catfish mucus. *Food Microbiology* 74: 143–50. doi: 10.1016/j.fm.2018.03.013.

Dixon, M., S. Flint, J. Palmer, R. Love, P. Biggs, and A. Beuger. 2018. Analysis of culturable and non-culturable bacteria and their potential to form biofilms in a primary treated dairy wastewater system. *Environmental Technology* 39 (17):2185–92. doi: 10.1080/09593330.2017.1352034.

Duanis-Assaf, D., D. Steinberg, Y. Chai, and M. Shemesh. 2016. The LuxS based quorum sensing governs lactose induced biofilm formation by *Bacillus subtilis*. *Frontiers in Microbiology* 6:1517. doi: 10.3389/fmicb.2015.01517.

Dubey, G. P., and S. Ben-Yehuda. 2011. Intercellular nanotubes mediate bacterial communication. *Cell* 144 (4):590–600. doi: 10.1016/j.cell.2011.01.015.

Dutra, T. V., M. D. S. Fernandes, M. R. F. G. Perdoncini, M. M. dos Anjos, and B. A. D. A. Filho. 2018. Capacity of *Escherichia coli* and *Staphylococcus* aureus to produce biofilm on stainless steel surfaces in the presence of food residues. *Journal of Food Processing and Preservation* 42 (4):e13574. doi: 10.1111/jfpp.13574.

Dygico, L. K., P. M. O'Connor, M. Hayes, C. G. M. Gahan, H. Grogan, and C. M. Burgess. 2019. *Lactococcus lactis* subsp. *lactis* as a natural anti-listerial agent in the mushroom industry. *Food Microbiology* 82: 30–5. doi: 10.1016/j.fm.2019.01.015.

Dzieciol, M., E. Schornsteiner, M. Muhterem-Uyar, B. Stessl, M. Wagner, and S. Schmitz-Esser. 2016. Bacterial diversity of floor drain biofilms and drain waters in a *Listeria monocytogenes* contaminated food processing environment. *International Journal of Food Microbiology* 223:33–40. doi: 10.1016/j.ijfoodmicro.2016.02.004.

Endersen, L., J. O'Mahony, C. Hill, R. P. Ross, O. McAuliffe, and A. Coffey. 2014. Phage therapy in the food industry. *Annual Review of Food Science and Technology* 5 (1):327–49. doi: 10.1146/annurev-food-030713-092415.

Fagerlund, A., T. Møretrø, E. Heir, R. Briandet, and S. Langsrud. 2017. Cleaning and disinfection of biofilms composed of *Listeria monocytogenes* and background microbiota from meat processing surfaces. *Applied and Environmental Microbiology* 83 (17):e01046–17. doi: 10.1128/AEM.01046-17.

Flemming, H. C., and J. Wingender. 2010. The biofilm matrix. *Nature Reviews Microbiology* 8 (9):623–33. doi: 10.1038/nrmicro2415.

Galié, S., C. García-Gutiérrez, E. M. Miguélez, C. J. Villar, and, and F. Lombó, 2018. Biofilms in the food industry: Health aspects and control methods. *Frontiers in Microbiology* 9:898. doi: 10.3389/fmicb.2018.00898.

Giaouris, E., N. Chorianopoulos, A. Doulgeraki, and G. J. Nychas. 2013. Co-culture with *Listeria monocytogenes* within a dual-species biofilm community strongly increases resistance of *Pseudomonas putida* to benzalkonium chloride. *PLoS One* 8 (10):e77276. doi: 10.1371/journal.pone.

Gomes, L. C., J. Deschamps, R. Briandet, and F. J. Mergulhão. 2018. Impact of modified diamond-like carbon coatings on the spatial organization and disinfection of mixed-biofilms composed of *Escherichia coli* and *Pantoea agglomerans* industrial isolates. *International Journal of Food Microbiology* 277:74–82. doi: 10.1016/j.ijfoodmicro.2018.04.017.

Gomes, M. Z. D. V., and M. Nitschke. 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 (2):441–7. doi: 10.1016/j.foodcont.2011.11.025.

Gomes, L. C., L. N. Silva, M. Simões, L. F. Melo, and F. J. Mergulhão. 2015. *Escherichia coli* adhesion, biofilm development and antibiotic susceptibility on biomedical materials. *Journal of Biomedical Materials Research Part A* 103 (4):1414–23. doi: 10.1002/jbm.a.35277.

Govaert, M., C. Smet, M. Baka, T. Janssens, and J. Van Impe. 2018. Influence of incubation conditions on the formation of model biofilms by *Listeria monocytogenes* and *Salmonella* Typhimurium on abiotic surfaces. *Journal of Applied Microbiology* 125 (6):1890–900. doi: 10.1111/jam.14071.

Guillonneau, R., C. Baraquet, A. Bazire, and M. Molmeret. 2018. Multispecies biofilm development of marine bacteria implies complex relationships through competition and synergy and modification of matrix components. *Frontiers in Microbiology* 9:1960. doi: 10.3389/fmicb.2018.01960.

Gule, N. P., N. M. Begum, and B. Klumperman. 2016. Advances in biofouling mitigation: A review. *Critical Reviews in Environmental Science and Technology* 46 (6):535–55. doi: 10.1080/10643389.2015.1114444.

Gutiérrez, D., L. Rodríguez-Rubio, B. Martínez, A, Rodríguez, and, and P. García, 2016. Bacteriophages as weapons against bacterial biofilms in the food industry. *Frontiers in Microbiology* 7:825. doi: 10.3389/fmicb.2016.00825.

Habimana, O., E. Heir, S. Langsrud, A. W. Åsli, and T. Møretrø. 2010. Enhanced surface colonization by *Escherichia coli* O157:H7 in biofilms formed by an *Acinetobacter calcoaceticus* isolate from meat-processing environments. *Applied and Environmental Microbiology* 76 (13):4557–9. doi: 10.1128/AEM.02707-09.

Hall-Stoodley, L., J. W. Costerton, and P. Stoodley. 2004. Bacterial biofilms: From the natural environment to infectious diseases. *Nature Reviews Microbiology* 2 (2):95–108. doi: 10.1038/nrmicro821.

Hansen, L. B. S., D. Ren, M. Burmølle, and S. J. Sørensen. 2017. Distinct gene expression profile of *Xanthomonas retroflexus* engaged in synergistic multispecies biofilm formation. *The ISME Journal* 11 (1):300–3. doi: 10.1038/ismej.2016.107.

Herschend, J., Z. B. V. Damholt, A. M. Marquard, B. Svensson, S. J. Sørensen, P. HäGglund, and, and M. Burmølle. 2017. A meta-proteomics approach to study the interspecies interactions affecting microbial biofilm development in a model community. *Scientific Reports* 7:16483. doi: 10.1038/s41598-017-16633-6.

Hossain, M. I., M. Sadekuzzaman, and S. D. Ha. 2017. Probiotics as potential alternative biocontrol agents in the agriculture and food

industries: A review. *Food Research International* 100:63–73. doi: 10.1016/j.foodres.2017.07.077.

Hussain, M. A., H. Liu, Q. Wang, F. Zhong, Q. Guo, and S. Balamurugan. 2017. Use of encapsulated bacteriophages to enhance farm to fork food safety. *Critical Reviews in Food Science and Nutrition* 57 (13):2801–10. doi: 10.1080/10408398.2015.1069729.

Hüwe, C., J. Schmeichel, F. Brodkorb, S. Dohlen, K. Kalbfleisch, M. Kreyenschmidt, R. Lorenz, and J. Kreyenschmidt. 2018. Potential of antimicrobial treatment of linear low-density polyethylene with poly((tert-butyl-amino)-methyl-styrene) to reduce biofilm formation in the food industry. *Biofouling* 34 (4):378–87. doi: 10.1080/08927014.2018.1453926.

Ibusquiza, P. S., M. N. Groot, A. Debán-Valles, T. Abee, and, and H. M. W. den Besten. 2015. Impact of growth conditions and role of *sigB* on *Listeria monocytogenes* fitness in single and mixed biofilms cultured with *Lactobacillus plantarum*. *Food Research International* 71:140–5. doi: 10.1016/j.foodres.2015.03.001.

Ibusquiza, P. S., J. J. R. Herrera, D. M. Vázquez-Sánchez, and, and M. L. Cabo, 2012. Adherence kinetics, resistance to benzalkonium chloride and microscopic analysis of mixed biofilms formed by *Listeria monocytogenes* and *Pseudomonas putida*. *Food Control* 25 (1):202–10. doi: 10.1016/j.foodcont.2011.10.002.

Ica, T., V. Caner, O. Istanbullu, H. D. Nguyen, B. Ahmed, D. R. Call, and H. Beyenal. 2012. Characterization of Mono- and mixed-culture *Campylobacter jejuni* biofilms. *Applied and Environmental Microbiology* 78 (4):1033–8. doi: 10.1128/AEM.07364-11.

Inaba, T., T. Ichihara, Y. Yawata, M. Toyofuku, H. Uchiyama, and N. Nomura. 2013. Three-dimensional visualization of mixed species biofilm formation together with its substratum. *Microbiology and Immunology* 57 (8):589–93. doi: 10.1111/1348-0421.12064.

Iñiguez-Moreno, M., M. Gutiérrez-Lomelí, P. J. Guerrero-Medina, and M. G. Avila-Novoa. 2018. Biofilm formation by *Staphylococcus aureus* and *Salmonella* spp. under mono and dual-species conditions and their sensitivity to cetrimonium bromide, peracetic acid and sodium hypochlorite. *Brazilian Journal of Microbiology* 49 (2):310–9. doi: 10.1016/j.bjm.2017.08.002.

Iñiguez-Moreno, M., M. Gutiérrez-Lomelí, and M. G. Avila-Novoa. 2019. Kinetics of biofilm formation by pathogenic and spoilage microorganisms under conditions that mimic the poultry, meat, and egg processing industries. *International Journal of Food Microbiology* 303:32–41. doi: 10.1016/j.ijfoodmicro.2019.04.012.

Jahid, I. K., N. R. Han, S. Srey, and S. D. Ha. 2014. Competitive interactions inside mixed-culture biofilms of *Salmonella* Typhimurium and cultivable indigenous microorganisms on lettuce enhance microbial resistance of their sessile cells to ultraviolet C (UV-C) irradiation. *Food Research International* 55:445–54. doi: 10.1016/j.foodres.2013.11.042.

Jindal, S., S. Anand, L. Metzger, and J. Amamcharla. 2018. A comparison of biofilm development on stainless steel and modified-surface plate heat exchangers during a 17-h milk pasteurization run. *Journal of Dairy Science* 101 (4):2921–6. doi: 10.3168/jds.2017-14028.

Kart, D., S. Tavernier, H. V. Acker, H. J. Nelis, and T. Coenye. 2014. Activity of disinfectants against multispecies biofilms formed by *Staphylococcus aureus*, *Candida albicans* and *Pseudomonas aeruginosa*. *Biofouling* 30 (3):377–83. doi: 10.1080/08927014.2013.878333.

Kerekes, E. B., É. Deák, M. Takó, R. Tserennadmid, T. Petkovits, C. Vágvolgyi, and J. Krisch. 2013. Anti-biofilm forming and anti-quorum sensing activity of selected essential oils and their main components on food-related micro-organisms. *Journal of Applied Microbiology* 115 (4):933–42. doi: 10.1111/jam.12289.

Kives, J., D. Guadarrama, B. Orgaz, A. Rivera-Sen, J. Vazquez, and C. SanJose. 2005. Interactions in biofilms of *Lactococcus lactis* ssp. *cremoris* and *Pseudomonas fluorescens* cultured in cold UHT milk. *Journal of Dairy Science* 88 (12):4165–71. doi: 10.3168/jds.S0022-0302(05)73102-7.

Knowles, J. R., S. Roller, D. B. Murray, and A. S. Naidu. 2005. Antimicrobial action of carvacrol at different stages of dual-species biofilm development by *Staphylococcus aureus* and *Salmonella enterica* Serovar Typhimurium. *Applied and Environmental Microbiology* 71 (2):797–803. doi: 10.1128/AEM.71.2.797–803.2005.

Kregiel. D. 2014. Advances in biofilm control for food and beverage industry using organo-silane technology: A review. *Food Control* 40: 32–40. doi: 10.1016/j.foodcont.2013.11.014.

Ksontini, H., F. Kachouri, and M. Hamdi. 2013. Dairy biofilm: Impact of microbial community on raw milk quality. *Journal of Food Quality* 36 (4):282–90. doi: 10.1111/jfq.12036.

Langsrud, S., B. Moen, T. Møretrø, M. Løype, and E. Heir. 2016. Microbial dynamics in mixed culture biofilms of bacteria surviving sanitation of conveyor belts in salmon-processing plants. *Journal of Applied Microbiology* 120 (2):366–78. doi: 10.1111/jam.13013.

Lee, K. W. K., S. Periasamy, M. Mukherjee, C. Xie, S. Kjelleberg, and S. A. Rice. 2014. Biofilm development and enhanced stress resistance of a model, mixed-species community biofilm. *The ISME Journal* 8 (4):894–907. doi: 10.1038/ismej.2013.194.

Lemos, M., A. Borges, J. Teodósio, P. Araújo, F. Mergulhão, L. Melo, an, and M. Simões, 2014. The effects of ferulic and salicylic acids on *Bacillus cereus* and *Pseudomonas fluorescens* single- and dual-species biofilms. *International Biodeterioration & Biodegradation* 86:42–51. doi: 10.1016/j.ibiod.2013.06.011.

Lemos, M., I. Gomes, F. Mergulhão, L. Melo, and M. Simões. 2015. The effects of surface type on the removal of *Bacillus cereus* and *Pseudomonas fluorescens* single and dual species biofilms. *Food and Bioproducts Processing* 93:234–41. doi: 10.1016/j.fbp.2014.08.009.

Lelieveld, H. L. M., J. Holah, and D. Napper. 2013. *Handbook of hygiene control in the food industry*. 2nd ed. Cambridge, UK: Woodhead Publishing.

Lequette, Y., G. Boels, M. Clarisse, and C. Faille. 2010. Using enzymes to remove biofilms of bacterial isolates sampled in the food-industry. *Biofouling* 26 (4):421–31. doi: 10.1080/08927011003699535.

Li, L., H. Meng, D. Gu, Y. Li, and M. Jia. 2019. Molecular mechanisms of *Vibrio parahaemolyticus* pathogenesis. *Microbiological Research* 222:43–51. doi: 10.1016/j.micres.2019.03.003.

Lim, E. S., J. E. Lee, J. S. Kim, and O. K. Koo. 2017. Isolation of indigenous bacteria from a cafeteria kitchen and their biofilm formation and disinfectant susceptibility. *LWT-Food Science and Technology* 77:376–82. doi: 10.1016/j.lwt.2016.11.060.

Lindsay, D., V. S. Brözel, J. F. Mostert, and A. von Holy. 2002. Differential efficacy of a chlorine dioxide-containing sanitizer against single species and binary biofilms of a dairy-associated *Bacillus cereus* and a *Pseudomonas fluorescens* isolate. *Journal of Applied Microbiology* 92 (2):352–61. doi: 10.1046/j.1365-2672.2002.01538.x.

Lindsay, D., and A. von Holy. 2006. What food safety professionals should know about bacterial biofilms. *British Food Journal* 108 (1): 27–37. doi: 10.1108/00070700610637616.

Liu, J., S. Yu, B. Han, and J. Chen. 2017. Effects of benzalkonium chloride and ethanol on dual-species biofilms of *Serratia liquefaciens* S1 and *Shewanella putrefaciens* S4. *Food Control* 78:196–202. doi: 10.1016/j.foodcont.2017.02.063.

Liu, N. T., G. R. Bauchan, C. B. Francoeur, D. R. Shelton, Y. M. Lo, and X. Nou. 2016a. *Ralstonia insidiosa* serves as bridges in biofilm formation by foodborne pathogens *Listeria monocytogenes*, *Salmonella enterica*, and Enterohemorrhagic *Escherichia coli*. *Food Control* 65:14–20. doi: 10.1016/j.foodcont.2016.01.004.

Liu, N. T., A. M. Lefcourt, X. Nou, D. R. Shelton, G. Zhang, and Y. M. Lo. 2013. Native microflora in fresh-cut produce processing plants and their potentials for biofilm formation. *Journal of Food Protection* 76 (5):827–32. doi: 10.4315/0362-028X.JFP-12-433.

Liu, W., H. L. Røder, J. S. Madsen, T. Bjarnsholt, S. J. Sørensen, and M. Burmølle. 2016b. Interspecific bacterial interactions are reflected in multispecies biofilm spatial organization. *Frontiers in Microbiology* 7:1366. doi: 10.3389/fmicb.2016.01366.

Liu, W., J. Russel, M. Burmølle, S. J. Sørensen, and J. S. Madsen. 2018. Micro-scale intermixing: A requisite for stable and synergistic co-establishment in a four-species biofilm. *The ISME Journal* 12 (8): 1940–51. doi: 10.1038/s41396-018-0112-2..

Lourenço, A., H. Machado, and L. Brito. 2011. Biofilms of *Listeria monocytogenes* produced at 12 °C either in pure culture or in co-culture with *Pseudomonas aeruginosa* showed reduced susceptibility to sanitizers. *Journal of Food Science* 76 (2):M143–8. doi: 10.1111/j.1750-3841.2010.02011.x.

Lu, T. K., and J. J. Collins. 2007. Dispersing biofilms with engineered enzymatic bacteriophage. *Proceedings of the National Academy of Sciences of the United States of America* 104 (27):11197–202. doi: 10.1073/pnas.0704624104.

Luo, Y., W. Yi, Y. Yao, N. Zhu, and P. Qin. 2018. Characteristic diversity and antimicrobial resistance of *Salmonella* from gastroenteritis. *Journal of Infection and Chemotherapy* 24 (4):251–5. doi: 10.1016/j.jiac.2017.11.003.

Machado, A., K. K. Jefferson, and N. Cerca. 2013. Interactions between *Lactobacillus crispatus* and bacterial vaginosis (BV)-associated bacterial species in initial attachment and biofilm formation. *International Journal of Molecular Sciences* 14 (6):12004–12. doi: 10.3390/ijms140612004.

Machado, I., S. P. Lopes, A. M. Sousa, and M. O. Pereira. 2012. Adaptive response of single and binary *Pseudomonas aeruginosa* and *Escherichia coli* biofilms to benzalkonium chloride. *Journal of Basic Microbiology* 52 (1):43–52. doi: 10.1002/jobm.201100137.

Madsen, J. S., M. Burmølle, L. H. Hansen, and S. J. Sørensen. 2012. The interconnection between biofilm formation and horizontal gene transfer. *FEMS Immunology & Medical Microbiology* 65 (2):183–95. doi: 10.1111/j.1574-695X.2012.00960.x.

Maes, S., M. Heyndrickx, T. Vackier, H. Steenackers, A. Verplaetse, and K. D. Reu. 2019. Identification and spoilage potential of the remaining dominant microbiota on food contact surfaces after cleaning and disinfection in different food industries. *Journal of Food Protection* 82 (2):262–75. doi: 10.4315/0362-028X.JFP-18-226.

Maifreni, M., F. Frigo, I. Bartolomeoli, S. Buiatti, S. Picon, and M. Marino. 2015. Bacterial biofilm as a possible source of contamination in the microbrewery environment. *Food Control* 50:809–14. doi: 10.1016/j.foodcont.2014.10.032.

Makovcova, J., V. Babak, P. Kulich, J. Masek, M. Slany, and L. Cincarova. 2017. Dynamics of mono- and dual-species biofilm formation and interactions between *Staphylococcus aureus* and gram-negative bacteria. *Microbial Biotechnology* 10 (4):819–32. doi: 10.1111/1751-7915.12705.

Marouani-Gadri, N., G. Augier, and B. Carpentier. 2009. Characterization of bacterial strains isolated from a beef-processing plant following cleaning and disinfection – Influence of isolated strains on biofilm formation by Sakaï and EDL 933 *E. coli* O157:H7. *International Journal of Food Microbiology* 133 (1–2):62–7. doi: 10.1016/j.ijfoodmicro.2009.04.028.

Meervenne, E. V., R. D. Weirdt, E. V. Coillie, F. Devlieghere, L. Herman, and N. Boon. 2014. Biofilm models for the food industry: Hot spots for plasmid transfer? *Pathogens and Disease* 70 (3):332–8. doi: 10.1111/2049-632X.12134.

Meireles, A., A. Borges, E. Giaouris, and M. Simões. 2016. The current knowledge on the application of anti-biofilm enzymes in the food industry. *Food Research International* 86:140–6. doi: 10.1016/j.foodres.2016.06.006.

Millezi, F. M., M. O. Pereira, N. N. Batista, N. Camargos, I. Auad, M. D. G. Cardoso, and R. H. Piccoli. 2012. Susceptibility of monospecies and dual-species biofilms of *Staphylococcus aureus* and *Escherichia coli* to essential oils. *Journal of Food Safety* 32 (3):351–9. doi: 10.1111/j.1745-4565.2012.00387.x.

Mizan, M. F. R., M. Ashrafudoulla, M. Sadekuzzaman, I. Kang, and S. D. Ha. 2018. Effects of NaCl, glucose, and their combinations on biofilm formation on black tiger shrimp (*Penaeus monodon*) surfaces by *Vibrio parahaemolyticus*. *Food Control* 89:203–9. doi: 10.1016/j.foodcont.2017.12.004.

Møretrø, M., and S. Langsrud. 2017. Residential bacteria on surfaces in the food industry and their implications for food safety and quality. *Comprehensive Reviews in Food Science and Food Safety* 16 (5):1022–41. doi: 10.1111/1541-4337.12283.

Nadell, C. D., K. Drescher, and K. R. Foster. 2016. Spatial structure, cooperation and competition in biofilms. *Nature Reviews Microbiology* 14 (9):589–600. doi: 10.1038/nrmicro2016.84.

Nahar, S., M. F. R. Mizan, A. J. Ha, and S. D. Ha. 2018. Advances and future prospects of enzyme-based biofilm prevention approaches in the food industry. *Comprehensive Reviews in Food Science and Food Safety* 17 (6):1484–502. doi: 10.1111/1541-4337.12382.

Nakamura, Y., N. Yamamoto, Y. Kino, N. Yamamoto, S. Kamei, H. Mori, K. Kurokawa, and N. Nakashima. 2016. Establishment of a multi-species biofilm model and metatranscriptomic analysis of biofilm and planktonic cell communities. *Applied Microbiology and Biotechnology* 100 (16):7263–79. doi: 10.1007/s00253-016-7532-6.

Nielsen, A. T., T. Tolker-Nielsen, K. B. Barken, and S. Molin. 2000. Role of commensal relationships on the spatial structure of a surface-attached microbial consortium. *Environmental Microbiology* 2 (1):59–68. doi: 10.1046/j.1462-2920.2000.00084.x..

Norwood, D. E., and A. Gilmour. 2000. The growth and resistance to sodium hypochlorite of *Listeria monocytogenes* in a steady-state multispecies biofilm. *Journal of Applied Microbiology* 88 (3):512–20. doi: 10.1046/j.1365-2672.2000.00990.x.

Pammi, M., R. Liang, J. Hicks, T. A. Mistretta, and J. Versalovic. 2013. Biofilm extracellular DNA enhances mixed species biofilms of *Staphylococcus epidermidis* and *Candida albicans*. *BMC Microbiology* 13 (1):257. doi: 10.1186/1471-2180-13-257.

Pang, X., and H. G. Yuk. 2018. Effect of *Pseudomonas aeruginosa* on the sanitizer sensitivity of *Salmonella* Enteritidis biofilm cells in chicken juice. *Food Control* 86:59–65. doi: 10.1016/j.foodcont.2017.11.012.

Pang, X., C. Wong, H. J. Chung, and H. G. Yuk. 2019. Biofilm formation of *Listeria monocytogenes* and its resistance to quaternary ammonium compounds in a simulated salmon processing environment. *Food Control* 98:200–8. doi: 10.1016/j.foodcont.2018.11.029.

Pang, X. Y., Y. S. Yang, and H. G. Yuk. 2017. Biofilm formation and disinfectant resistance of *Salmonella* sp. in mono- and dual-species with *Pseudomonas aeruginosa*. *Journal of Applied Microbiology* 123 (3):651–60. doi: 10.1111/jam.13521.

Parijs, I., and H. P. Steenackers. 2018. Competitive inter-species interactions underlie the increased antimicrobial tolerance in multispecies brewery biofilms. *The ISME Journal* 12 (8):2061–75. doi: 10.1038/s41396-018-0146-5.

Patange, A., D. Boehm, D. Ziuzina, P. J. Cullen, B. Gilmore, and P. Bourke. 2019. High voltage atmospheric cold air plasma control of bacterial biofilms on fresh produce. *International Journal of Food Microbiology* 293:137–45. doi: 10.1016/j.ijfoodmicro.2019.01.005.

Peck, O. P. W., Y. M. J. Chew, and M. R. Bird. 2019. On-line quantification of thickness and strength of single and mixed species biofilm grown under controlled laminar flow conditions. *Food and Bioproducts Processing* 113:49–59. doi: 10.1016/j.fbp.2018.08.009.

Pei, R., and G. R. Lamas-Samanamud. 2014. Inhibition of biofilm formation by T7 bacteriophages producing quorum-quenching enzymes. *Applied and Environmental Microbiology* 80 (17):5340–8. doi: 10.1128/AEM.01434-14.

Pignata, C., D. D'Angelo, E. Fea, and G. Gilli. 2017. A review on microbiological decontamination of fresh produce with nonthermal plasma. *Journal of Applied Microbiology* 122 (6):1438–55. doi: 10.1111/jam.13412.

Ramamurthy, T., A. Ghosh, G. P. Pazhani, and S. Shinoda. 2014. Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria. *Frontiers in Public Health* 2:103. doi: 10.3389/fpubh.2014.00103.

Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Swerdlow. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerging Infectious Diseases* 11 (4):603–9. doi: 10.3201/eid1104.040739.

Ren, D., J. S. Madsen, C. I. de la Cruz-Perera, L. Bergmark, S. J. Sørensen, and M. Burmølle. 2014. High-throughput screening of multispecies biofilm formation and quantitative PCR-based assessment of individual species proportions, useful for exploring interspecific bacterial interactions. *Microbial Ecology* 68 (1):146–54. doi: 10.1007/s00248-013-0315-z.

Rendueles, O., and J. M. Ghigo. 2012. Multi-species biofilms: How to avoid unfriendly neighbors. *FEMS Microbiology Reviews* 36 (5):972–89. doi: 10.1111/j.1574-6976.2012.00328.x.

Rice, S. A., S. Wuertz, and S. Kjelleberg. 2016. Next-generation studies of microbial biofilm communities. *Microbial Biotechnology* 9 (5):677–80. doi: 10.1111/1751-7915.12390.

Rickard, A. H., R. J. Palmer, Jr., D. S. Blehert, S. R. Campagna, M. F. Semmelhack, P. G. Egland, B. L. Bassler, and P. E. Kolenbrander. 2006. Autoinducer 2: A concentration-dependent signal for mutualistic bacterial biofilm growth. *Molecular Microbiology* 60 (6): 1446–56. doi: 10.1111/j.1365-2958.2006.05202.x.

Røder, H. L., P. K. Raghupathi, J. Herschend, A. Brejnrod, S. Knøchel, S. J. Sørensen, and M. Burmølle. 2015. Interspecies interactions result in enhanced biofilm formation by co-cultures of bacteria isolated from a food processing environment. *Food Microbiology* 51: 18–24. doi: 10.1016/j.fm.2015.04.008.

Rodríguez-Lopez, P., and M. L. Cabo. 2017. Tolerance development in *Listeria monocytogenes-Escherichia coli* dual-species biofilms after sublethal exposures to pronase-benzalkonium chloride combined treatments. *Food Microbiology* 67:58–66. doi: 10.1016/j.fm.2017.06.002.

Rodríguez-López, P., A. Carballo-Justo, L. A. Draper, and M. L. Cabo. 2017. Removal of *Listeria monocytogenes* dual-species biofilms using combined enzyme-benzalkonium chloride treatments. *Biofouling* 33 (1):45–58. doi: 10.1080/08927014.2016.1261847.

Russel, J., H. L. Røder, J. S. Madsen, M. Burmølle, and S. J. Sørensen. 2017. Antagonism correlates with metabolic similarity in diverse bacteria. *Proceedings of the National Academy of Sciences of the United States of America* 114 (40):10684–8. doi: 10.1073/pnas.1706016114.

Shen, C., Y. Luo, X. Nou, G. Bauchan, B. Zhou, Q. Wang, and P. Millner. 2012. Enhanced inactivation of *Salmonella* and *Pseudomonas* biofilms on stainless steel by use of T-128, a fresh-produce washing aid, in chlorinated wash solutions. *Applied and Environmental Microbiology* 78 (19):6789–98. doi: 10.1128/AEM.01094-12.

Shpigel, N. Y., Z. Pasternak, G. Factor, and Y. Gottlieb. 2015. Diversity of bacterial biofilm communities on sprinklers from dairy farm cooling systems in Israel. *PLoS One* 10 (9):e0139111. doi: 10.1371/journal.pone.0139111.

Si, X., and X. Quan. 2017. Prevention of multi-species wastewater biofilm formation using vanillin and EPS disruptors through non-microbicidal mechanisms. *International Biodeterioration & Biodegradation* 116:211–8. doi: 10.1016/j.ibiod.2016.11.009.

Simões, L. C., M. Lemos, P. Araújo, A. M. Pereira, and M. Simões. 2011. The effects of glutaraldehyde on the control of single and dual biofilms of *Bacillus cereus* and *Pseudomonas fluorescens*. *Biofouling* 27 (3):337–46. doi: 10.1080/08927014.2011.575935.

Simmons, M., K. Drescher, C. D. Nadell, and V. Bucci. 2018. Phage mobility is a core determinant of phage–bacteria coexistence in biofilms. *The ISME Journal* 12:532–43. doi: 10.1038/ismej.2017.190.

Skarp, C. P. A., M.-L. Hänninen, and H. I. K. Rautelin. 2016. Campylobacteriosis: The role of poultry meat. *Clinical Microbiology and Infection* 22 (2):103–9. doi: 10.1016/j.cmi.2015.11.019..

Somerton, B., D. Lindsay, J. Palmer, J. Brooks, and S. Flint. 2015. Changes in sodium, calcium, and magnesium ion concentrations that inhibit *Geobacillus* biofilms have no effect on *Anoxybacillus flavithermus* biofilms. *Applied and Environmental Microbiology* 81 (15): 5115–22. doi: 10.1128/AEM.01037-15.

Srey, S., I. K. Jahid, and S. D. Ha. 2013. Biofilm formation in food industries: A food safety concern. *Food Control* 31 (2):572–85. doi: 10.1016/j.foodcont.2012.12.001.

Strathmann, M., K. H. Mittenzwey, G. Sinn, W. Papadakis, and H. C. Flemming. 2013. Simultaneous monitoring of biofilm growth, microbial activity, and inorganic deposits on surfaces with an *in situ*, online, real-time, non-destructive, optical sensor. *Biofouling* 29 (5): 573–83. doi: 10.1080/08927014.2013.791287.

Sutherland, I. W., K. A. Hughes, L. C. Skillman, and T. Tait. 2004. The interaction of phage and biofilms. *FEMS Microbiology Letters* 232 (1):1–6. doi: 10.1016/S0378-1097(04)00041-2.

Tan, Y., M. Leonhard, D. Moser, S. Ma, and B. Schneider-Stickler. 2018. Inhibitory effect of probiotic lactobacilli supernatants on single and mixed non-albicans *Candida* species biofilm. *Archives of Oral Biology* 85:40–5. doi: 10.1016/j.archoralbio.2017.10.002.

Tang, L., S. Pillai, N. P. Revsbech, A. Schramm, C. Bischoff, and R. L. Meyer. 2011. Biofilm retention on surfaces with variable roughness and hydrophobicity. *Biofouling* 27 (1):111–21. doi: 10.1080/08927014.2010.544848.

Tang, X., S. H. Flint, J. D. Brooks, and R. J. Bennett. 2009. Factors affecting the attachment of micro-organisms isolated from ultrafiltration and reverse osmosis membranes in dairy processing plants. *Journal of Applied Microbiology* 107 (2):443–51. doi: 10.1111/j.1365-2672.2009.04214.x.

Uhlich, G. A., D. P. Rogers, and D. A. Mosier. 2010. *Escherichia coli* serotype O157:H7 retention on solid surfaces and peroxide resistance is enhanced by dual-strain biofilm formation. *Foodborne Pathogens and Disease* 7 (8):935–43. doi: 10.1089/fpd.2009.0503.

Ultee, A., E. P. W. Kets, and E. J. Smid. 1999. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. *Applied and Environmental Microbiology* 65 (10):4606–10.

Vale, J., M. Ribeiro, A. C. Abreu, I. Soares-Silva, and M. Simões. 2019. The use of selected phytochemicals with EDTA against *Escherichia coli* and *Staphylococcus epidermidis* single- and dual-species biofilms. *Letters in Applied Microbiology* 68 (4):313–20. doi: 10.1111/lam.13137.

Valera, M. J., M. J. Torija, A. Mas, and E. Mateo. 2015. Acetic acid bacteria from biofilm of strawberry vinegar visualized by microscopy and detected by complementing culture-dependent and culture-independent techniques. *Food Microbiology* 46:452–62. doi: 10.1016/j.fm.2014.09.006.

van der Veen, S., and T. Abee. 2011. Mixed species biofilms of *Listeria monocytogenes* and *Lactobacillus plantarum* show enhanced resistance to benzalkonium chloride and peracetic acid. *International Journal of Food Microbiology* 144 (3):421–31. doi: 10.1016/j.ijfoodmicro.2010.10.029.

Van Houdt, R., and C. W. Michiels. 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. *Journal of Applied Microbiology* 109 (4):1117–31. doi: 10.1111/j.1365-2672.2010.04756.x.

Varga, L., and J. Szigeti. 2016. Use of ozone in the dairy industry: A review. *International Journal of Dairy Technology* 69 (2):157–68. doi: 10.1111/1471-0307.12302.

Veluz, G. A., S. Pitchiah, and C. Z. Alvarado. 2012. Attachment of *Salmonella* serovars and *Listeria monocytogenes* to stainless steel and plastic conveyor belts. *Poultry Science* 91 (8):2004–10. doi: 10.3382/ps.2011-01689.

Verastegui, Y., J. K. Cheng, D. Engel, S. Kolczynski, J. Mortimer, J. Lavigne, T. Montalibet, M. Romantsov, B. J. Hall, D. R. McConkey, et al. 2014. Multisubstrate isotope labeling and metagenomic analysis of active soil bacterial communities. *mBio* 5 (4):e01157–14. doi: 10.1128/mBio.01157-14.

Vidakovic, L., P. K. Singh, R. Hartmann, C. D. Nadell, and K. Drescher. 2018. Dynamic biofilm architecture confers individual and collective mechanisms of viral protection. *Nature Microbiology* 3 (1): 26–31. doi: 10.1038/s41564-017-0050-1.

Vilanova, C., A. Iglesias, and M. Porcar. 2015. The coffee-machine bacteriome: Biodiversity and colonisation of the wasted coffee tray leach. *Scientific Reports* 5:17163. doi: 10.1038/srep17163.

Wang, R., N. Kalchayanand, and J. L. Bono. 2015. Sequence of colonization determines the composition of mixed biofilms by *Escherichia coli* O157:H7 and O111:H8 strains. *Journal of Food Protection* 78 (8):1554–9. doi: 10.4315/0362-028X.JFP-15-009.

Wang, R., N. Kalchayanand, J. W. Schmidt, and D. M. Harhay. 2013. Mixed biofilm formation by Shiga toxin-producing *Escherichia coli* and *Salmonella enterica* Serovar Typhimurium enhanced bacterial resistance to sanitization due to extracellular polymeric substances. *Journal of Food Protection* 76 (9):1513–22. doi: 10.4315/0362-028X.JFP-13-077.

Wang, X., X. Li, and J. Ling. 2017. *Streptococcus gordonii* LuxS/autoinducer-2 quorum-sensing system modulates the dual-species biofilm formation with *Streptococcus mutans*. *Journal of Basic Microbiology* 57 (7):605–16. doi: 10.1002/jobm.201700010.

Winkelströter, L. K., F. B. d. R. Teixeira, E. P. Silva, V. F. Alves, and E. C. P. De Martinis. 2014. Unraveling microbial biofilms of importance for food microbiology. *Microbial Ecology* 68 (1):35–46. doi: 10.1007/s00248-013-0347-4.

Winkelströter, L. K., F. L. Tulini, and E. C. P. De Martinis. 2015. Identification of the bacteriocin produced by cheese isolate *Lactobacillus paraplantarum* FT259 and its potential influence on *Listeria monocytogenes* biofilm formation. *LWT - Food Science and Technology* 64 (2):586–92. doi: 10.1016/j.lwt.2015.06.014.

Woo, J., and J. Ahn. 2013. Probiotic-mediated competition, exclusion and displacement in biofilm formation by food-borne pathogens. *Letters in Applied Microbiology* 56 (4):307–13. doi: 10.1111/lam.12051.

Xue, T., X. Chen, and F. Shang. 2014. Effects of lactose and milk on the expression of biofilm-associated genes in *Staphylococcus aureus* strains isolated from a dairy cow with mastitis. *Journal of Dairy Science* 97 (10):6129–34. doi: 10.3168/jds.2014-8344.

Yasunaga, A., A. Yoshida, K. Morikawa, K. Maki, S. Nakamura, I. Soh, S. Awano, and T. Ansai. 2013. Monitoring the prevalence of viable and dead cariogenic bacteria in oral specimens and *in vitro* biofilms by qPCR combined with propidium monoazide. *BMC Microbiology* 13 (1):157. doi: 10.1186/1471-2180-13-157.

Yuan, L., M. Burmølle, F. A. Sadiq, N. Wang, and G. He. 2018a. Interspecies variation in biofilm-forming capacity of psychrotrophic bacterial isolates from Chinese raw milk. *Food Control* 91:47–57. doi: 10.1016/j.foodcont.2018.03.026.

Yuan, L., F. A. Sadiq, M. Burmølle, T. Liu, and G. He. 2018b. Insights into bacterial milk spoilage with particular emphasis on the roles of heat-stable enzymes, biofilms, and quorum sensing. *Journal of Food Protection* 81 (10):1651–60. doi: 10.4315/0362-028X.JFP-18-094.

Zhao, J., Y. Shen, M. Haapasalo, Z. Wang, and Q. Wang. 2016. A 3D numerical study of antimicrobial persistence in heterogeneous multi-species biofilms. *Journal of Theoretical Biology* 392:83–98. doi: 10.1016/j.jtbi.2015.11.010.

Zhu, J., Y. Yan, Y. Wang, and D. Qu. 2019. Competitive interaction on dual-species biofilm formation by spoilage bacteria, *Shewanella baltica* and *Pseudomonas fluorescens*. *Journal of Applied Microbiology* 126 (4):1175–86. doi: 10.1111/jam.14187.

Zou, M., and D. Liu. 2018. A systematic characterization of the distribution, biofilm-forming potential and the resistance of the biofilms to the CIP processes of the bacteria in a milk powder processing factory. *Food Research International* 113:316–26. doi: 10.1016/j.foodres.2018.07.020.

Zupančič, J., P. M. Raghupathi, K. Houf, M. Burmølle, S. J. Sørensen, and N. Gunde-Cimerman. 2018. Synergistic interactions in microbial biofilms facilitate the establishment of opportunistic pathogenic fungi in household dishwashers. *Frontiers in Microbiology* 9:21. doi: 10.3389/fmicb.2018.00021.