J. Dairy Sci. 108:8101–8119
https://doi.org/10.3168/jds.2024-25397

Biofilm Formation in Dairy: A Food Safety Concern—Microbial community tracking from dairy farm to factory; Insights on biofilm management for enhanced food safety and quality

Gisèle LaPointe,[1]* Tara Wilson,[1] Armin Tarrah,[1] Mérilie Gagnon,[2]† and Denis Roy[2]
[1]Dairy at Guelph, University of Guelph, Guelph, ON, Canada, N1G 2W1
[2]Département des sciences des aliments, Université Laval, Québec, QC, Canada, G1K 7P4

ABSTRACT

This review aimed to assess the scope of the literature on tracking the microbial community of biofilms, focusing on the dairy farm and processing environments. The majority of studies focused on either production, storage, transport, or processing of milk, and 5 studies combined the investigation of both production and processing facilities. Factors influencing short-term changes in dairy microbiota, such as the occurrence of mastitis and season, were distinguished from factors revealed through long-term studies, such as feed and weather, rather than the milking equipment. Knowledge gaps were identified in relation to the study design, methods, data analysis, and interpretation. The application of DNA sequencing technologies is particularly challenging with respect to samples with low microbial load (milk, swabs). There are few studies on the microbial composition of in situ biofilms, which might require new technologies for detection before sampling. Fundamental studies on the structure of biofilms are needed to identify the on-farm practices affecting the cycle of biofilm development in milking systems.
Key words: dairy, biofilm, track, microbiota

INTRODUCTION

Food safety and quality rely on management of physical, chemical, and microbial risks in food production from farm to factories. This literature review examines current research on tracking microbial communities throughout the dairy supply chain, with a particular focus on microbial biofilms. Significant aspects of microbial community tracking, such as agricultural practices, food processing, and risk management, are explored in relation to biofilm formation and control. The review highlights the literature available on emerging technologies and methodologies for the detection and management of biofilms in food production environments. By scoping the existing literature, this review provides insights into the gaps in knowledge of microbial community tracking and biofilm management that could be addressed to continue improving food safety and quality.

Background

Pasteurization, cleaning, and sanitation measures in dairy production and processing facilities are designed to eliminate most pathogenic and spoilage bacteria (Rankin et al., 2017). However, adhered microorganisms can pose a risk to dairy production due to their ability to form biofilms on equipment surfaces and release bacteria to continue spreading through the production line. This can have detrimental effects on downstream processes, causing blockages, insufficient heat transfer, and erosion of milking and processing equipment, in addition to affecting product shelf life and safety (Seale et al., 2015).

General Concepts of Biofilm Formation in the Dairy Environment

Biofilms have been described as matrix-enclosed sessile populations of microbes that can be metabolically active (Costerton, 2004) and enable bacteria to persist within an environment. The 5 classic phases of biofilm formation are reversible adhesion, irreversible binding to the surface, microcolony formation, maturation, and dispersal (Marchand et al., 2012; Kostakioti et al., 2013; Crouzet et al., 2014). Progression through these steps depends on the gradient of hydrodynamic force, as well as nutrient levels, pH, ionic strength, and temperature (Kostakioti et al., 2013). During the production and processing of

Received July 7, 2024.
Accepted December 6, 2024.
*Corresponding author: glapoint@uoguelph.ca
†Current address: Centre ACER, Saint-Norbert-d'Arthabaska, QC, Canada, G0P 1B0.

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

milk and dairy products, routine sanitation imposes a further limitation on the dynamics of biofilm development. Previous studies have investigated well-known biofilm-forming bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* within the context of the food processing environment (Carrascosa et al., 2021). The induction of biofilm formation for many bacteria is environmentally driven and can be caused by microbial signaling (quorum sensing), nutrient availability, the use of antibacterial agents during cleaning, temperature, and pH (López et al., 2010; Liu et al., 2020). When bacteria can accumulate within the milking systems and processing facilities, alongside selective pressures such as temperature and time, this can lead to a shift within the milk microbiota, thus affecting production yield and quality (Falardeau et al., 2019).

During reversible attachment, bacteria are only weakly bound to the surface and can return to their planktonic state (Fu et al., 2021). During this attachment step, bacteria can effectively be removed by sanitizers (Rosado de Castro et al., 2017). Biofilm formation allows bacteria to evade these measures, particularly in concert with material corrosion, aging gaskets and hoses which provide shielding from shear forces and chemicals. Rosado de Castro et al. (2017) found that *Enterococcus faecium* and *Enterococcus faecalis* could form biofilms on stainless steel surfaces in a cheese production facility within 1 to 8 d of contact when the temperature ranged between 12°C and 47°C and between 10°C and 43°C, respectively. Furthermore, Diarra et al. (2023) demonstrated the capacity of 6 thermoresistant species, including the spoilage and potential pathogen *P. aeruginosa*, to form multispecies dairy biofilms on stainless steel in a Centers for Disease Control (CDC) biofilm reactor (licensed from the CDC; https://archive.epa.gov/pesticides/labs/web/pdf/handouts-from-biofilm-lab-session.pdf) fed microfiltered milk. Both monospecies or multispecies aggregations can be found in biofilms, which can pose challenges to the dairy industry due to the increased incidence of antimicrobial resistance and the potential to cause severe foodborne diseases seen with multispecies biofilms (Kim et al., 2022). The timing of biofilm formation should be contextualized for the practical cycles in both dairy production and processing environments. In the context of milking equipment, parlors are cleaned twice daily after each milking, whereas robots may have more frequent cleaning scheduled throughout the day. For processing plants, the cleaning and sanitation between production cycles depends on the length of the production run; the longer the cycle, the more risk of developing organic deposits which attract adherent bacteria.

In the dairy industry, biofilms can form on a variety of surfaces, including abiotic materials such as stainless steel, rubber, glass, and plastics such as polytetrafluoroethylene (**PTFE**) and polyvinyl chloride, as well as biotic surfaces such as the gastrointestinal epithelium and teats (Storgards et al., 1999; O'Toole et al., 2000; Jefferson, 2004; Weber et al., 2019; Du et al., 2020). These interfaces can be air-liquid, air-solid, or solid-liquid, each providing a niche for microbes according to their tolerance for oxygen.

Among the many factors that play a substantial role in the ability of bacterial colonies to form successful biofilms on surfaces within the dairy environment, the most cited are surface properties, which include hydrophobicity and extracellular matrix production (Ayhan et al., 2019). During the reversible stage of biofilm formation, adhesion to surfaces is categorized by van der Waals forces and hydrophobic interactions due to the innate negative charges of most bacteria (Dunne, 2002). Lipopolysaccharides of gram-negative bacteria and teichoic acids on the surface of gram-positive bacteria contribute to the net negative charges, which in turn facilitate the initial stages of bacterial adhesion (Ruhal and Kataria, 2021). Initial attachment can be supported by surface conditioning with milk proteins and organic residue, which allow bacteria to adhere (Flint et al., 2020).

In addition to these initial adhesion factors, another important process, called quorum sensing (**QS**), plays a significant role in biofilm development, both for gram-negative and gram-positive bacteria. This cell-to-cell communication system is based on signaling molecules (autoinducers) released by bacteria as their population density increases. Gram-negative bacteria utilize acyl-homoserine lactones as autoinducers, whereas autoinducing peptides are produced by gram-positive bacteria. This variation in QS molecules likely contributes to the observed differences in biofilm development between these 2 bacterial groups (Ruhal and Kataria, 2021).

Biofilms pose a significant challenge in several industries, including the dairy industry. Their persistence, due to a combination of factors, is of particular concern in environments with fluctuating oxygen levels. Although some bacteria thrive in aerobic conditions, such *E. coli* and *P. aeruginosa* (Colón-González et al., 2004; O'May et al., 2009), others prefer anaerobic environments (Doyle et al., 2015) This adaptability allows biofilms to persist under varying oxygen conditions. *Bacillus* biofilms pose a persistent challenge in the dairy industry, particularly in environments with fluctuating oxygen levels (Shemesh and Ostrov, 2020). *Bacillus* species can form heat-resistant spores, which are particularly prevalent in biofilms. These spores can withstand harsh environmental conditions, including fluctuating oxygen levels, further enhancing *Bacillus* persistence. Spore formation in *Bacillus* biofilms is often triggered by oxygen limitation, suggesting a role for oxygen-sensing mechanisms in this process (Härtig and

Jahn, 2012; Pisithkul et al., 2019). By integrating an understanding of oxygen dynamics, biofilm composition, and QS, the dairy industry can move to more effective control measures to ensure the production of high quality and safe products.

Objective of the Scoping Review

The purpose of this scoping review is to map the features of the existing literature on biofilms to identify the gaps in research pertaining specifically to the dairy environment. The concept of this scoping review is to determine whether the designs and frameworks of dairy biofilm research have addressed the monitoring or tracking of the microbial communities of biofilms across the dairy production and processing chain. The key objectives are to describe the range of topics and identify specific knowledge gaps in the literature using systematic methodology (Peters et al., 2015).

LITERATURE REVIEW METHODS

Search Terms and Strategy

The PRISMA 2020 guidelines were applied to the selection of citations (Page et al., 2021), as supported for systematic scoping reviews by Peters et al. (2015). Two reviewers performed the search strategy independently using 5 databases accessed through the 2 following interfaces: Web of Science (3 databases: Science Citation Index Expanded, the Emerging Sources Citation Index, and the Conference Proceedings Citation Index–Science; https://www.webofscience.com) and ProQuest (2 databases: Biological Science Collection and Agricultural and Environmental Science Collections; https://www.proquest.com/).

Information Sources and Search Structure

The strategy consisted of searching each interface independently to use automatic filtering where possible, with sets of keywords joined by Boolean operators (details of the search strings can be found in the Supplemental Material S1, see Notes). The keywords for topics included dairy and biofilm combined with agriculture, farm, food, food production, food processing, dairy biofilm, dairy food, dairy plant, and milking equipment. Types of citations that were included were articles, reviews, proceedings papers, and early access, to cover both published and unpublished (gray) literature, and language was restricted to English. Types of citations that were excluded were editorial material and corrections, as well as studies pertaining to disease. Year of publication was restricted to between 1992 to May 2024.

Screening and Data Collection

Elimination of 100 duplicates and application of the inclusion and exclusion criteria during screening of title, abstract, and full text were carried out in the Covidence environment (https://www.covidence.org) independently by 2 reviewers. The exclusion criteria applied during screening of citations were topics such as in vitro modeling of adhesion and biofilm control strategies, and in vitro testing of biofilm-forming ability on any surfaces. One reviewer compiled the citation data, which was examined by a second reviewer and validated by a third reviewer. The included citations were exported from Covidence to a Microsoft Excel spreadsheet for compilation of terms found in the title and abstract. Quantitative and qualitative analyses were performed on the classification of the content of the citations. Full text review was then carried out to select records pertinent to source tracking or monitoring the microbial community of biofilms in the farm and processing environments, excluding any record that did not contain the keywords “biofilm” and “track” or “monitor.”

RESULTS

Study Characteristics

A total of 626 records passed the title and abstract screening stage from the combined searches, after excluding 100 duplicates and 260 records as irrelevant (Figure 1). During verification for eligibility, a further 268 records were excluded using the criteria, leaving 358 records for full text review and compilation of topics (Figure 1; Supplemental Material S2, see Notes). From 1992 to 2024, over half of the records (55% of 358) were published since 2019. Out of 43 literature reviews, 21 were published in the past 5 years, including one systematic (Zou and Liu, 2018) and one semi-systematic review (Joshi et al., 2022). Additional details on the distribution of the topics of the 358 records are available in Supplemental Figure S1 (see Notes).

Compilation of Final Selection of Eligible Studies for Review

Screening of the full text of the articles further refined record selection to studies pertaining in part or entirely to monitoring the microbial community either in spatial or temporal axes in the farm or processing environments, which included surface sampling after cleaning. Studies focused on raw milk or processed products generally inferred biofilm formation among the list of possible factors that could affect their results. In vitro studies of biofilm formation were excluded, unless relevant to

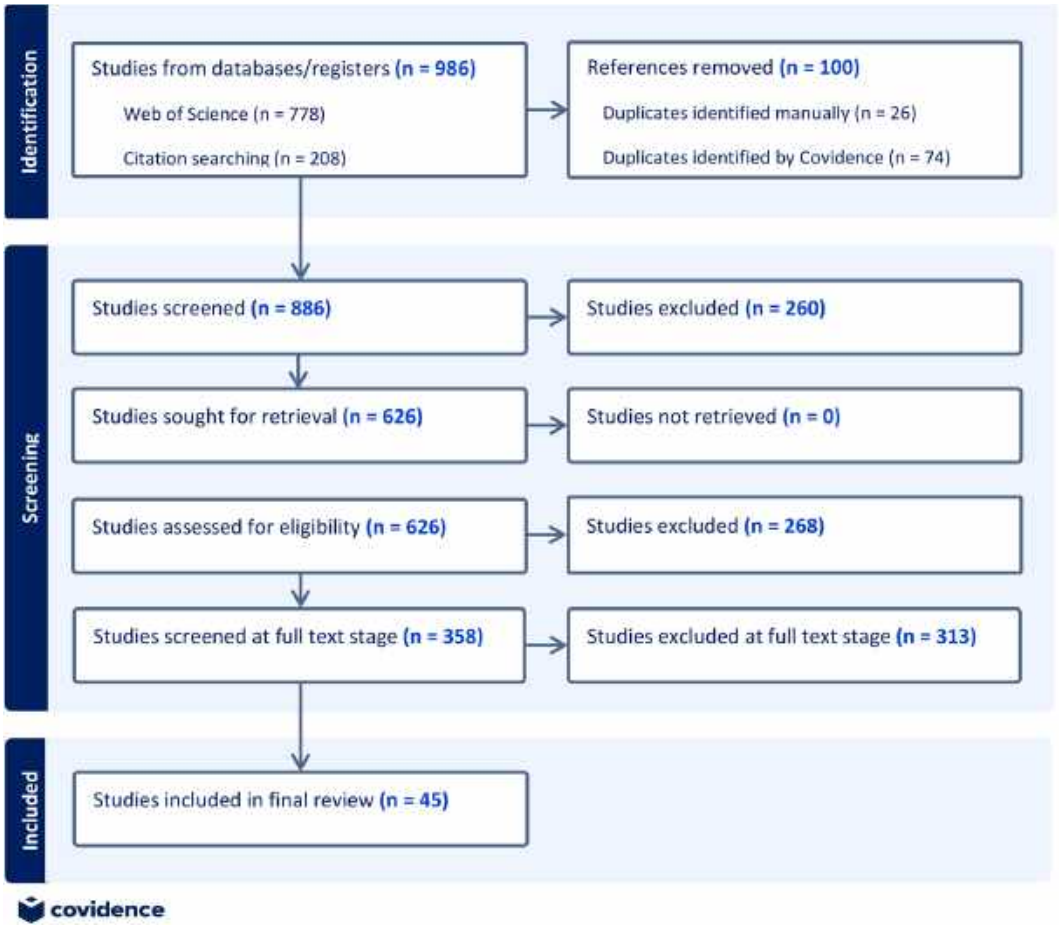

Figure 1. Flowchart of the database search (1992–2024) and screening process used in this scoping review. Records pertaining to in vitro modeling of adhesion and biofilm control strategies, and in vitro testing of biofilm-forming ability on any surfaces were excluded. Any record not containing the key word "biofilm," "track," or "monitor" in the full text was excluded.

factors determining the frequency of occurrence of the phenotype in specific microbial niches. A total of 316 out of 358 records were excluded due to lack of relevance to the inclusion criteria of tracking, monitoring or profiling microbial communities in the dairy production or processing environments. Many of the excluded studies were focused on obtaining and screening isolates in vitro for biofilm formation without referring to any explanatory factors from the environment. This resulted in a targeted list of 45 extracted studies specifically mentioning biofilms in relation to microbial community tracking in dairy production and processing, which include 11 reviews (Table 1) and a total of 34 studies (Tables 1, 2, 3, and 4). Five publications covered both the production and processing facilities, 15 focused on production, milk collection, storage, and transport; and 14 reports concerned the processing environment and products (Tables 1, 2, 3, and 4). None of the extracted studies overlap those reviewed by Flint et al. (2020). Other comprehensive reviews and studies exist within this time frame on sanitation or the microbiota of milk and dairy products that do not specifically mention biofilms, and so have not been included in the systematic approach taken here, except for 4 that are used in the discussion to support analyses of the risk factors pertaining to the dairy environment (Elmoslemany et al., 2009, 2010; Ouamba et al., 2020, 2022) and 4 citations to support risk analysis and management strategies (Fischer et al., 2012; Enayaty-Ahangar et al., 2021; Rey-Cadilhac et al., 2021, 2023).

Out of the 11 reviews, 2 pertain to cleaning and sanitation in the dairy industry, both specifically mentioning biofilms (Vlková et al., 2008; Rankin et al., 2017; Table 1). One review focused on biofilms on-farm (Butucel et al., 2022), 5 on combined farm and processing (Marchand et al., 2012; Machado et al., 2017; Flint et al., 2020; Castro et al., 2022; Joshi et al., 2022), 1 on psychrotrophic bacteria in raw milk (Yuan et al., 2019), and 1 focused on reviewing the applications of multiomics methods for analyzing dairy biofilms (Yuan et al., 2023). Finally, one last review addressed the application of beneficial

Table 1. Extracted peer-reviewed studies related to tracking biofilms in the dairy production and processing environments: reviews (n = 11) and combined environments (n = 5)

Location and product	Microorganism and methods	Reference	Highlights of the study	Conclusion
Reviews				
Biofilms on farm	Pathogens (multiple methods)	Butucel et al. (2022)	Antibiofilm intervention strategies	Antimicrobial resistance in biofilms
Farm to fork	Super-shedders (multiple methods)	Castro et al. (2022)	Biofilms in recto-anal junction, possible link to outbreak	High load events can exceed control measures
Biofilms on farm and processing	Spoilage and specific pathogens	Flint et al. (2020)	Comprehensive review of progress over 20 years	Gaps in research, promise of quantitative models
Biofilms in housing	LAB and pathogens	Guéneau et al. (2022)	Pathogen exclusion using biofilm design	Gaps in research for using positive biofilms
Microbial risk assessment	Pathogens and spoilage; multiple	Joshi et al. (2022)	Multiomics contributes to precision food safety	Recovery of pathogens a persisting problem
Farm to processing facility	Pathogens and spoilage; multiple	Machado et al. (2017)	Cold storage and heat-stable enzymes	Screening could help direct milk to process
Production and processing	Microbiota	Marchand et al. (2012)	Covers basics, CIP, and biofilm modeling	Need for coating strategies and fortified sanitizers
Processing	Pathogens and spoilage	Rankin et al. (2017)	Progress in sanitation over 100 years in the US	Technological challenges for the future
Biofilms in the dairy industry	Microbes culture-dependent	Vlková et al. (2008)	Wide review of sanitation agents and testing methods	Standardization is needed for testing sanitation processes
Raw milk	Psychrotrophs (culture)	Yuan et al. (2019)	Technological problems; heat-stable enzymes	Biofilms as persistent sources of contamination
Dairy biofilms	Multiple methods	Yuan et al. (2023)	Multiomics describe biofilm phenotypes	Biofilm control strategies
Combined				
Small-scale cheese facility	*Bacillus cereus* (culture)	Cruz-Facundo et al. (2023)	87.5% of isolates formed pellicle biofilms on glass	Cheese and air isolates were similar
Artisanal cheese	*Staphylococcus aureus* (culture)	Gajewska et al. (2022)	62% of isolates were strong biofilm formers	Raw milk cheese is at risk for *S. aureus*
Bulk tank milk to milk powder	Microbiota (16S and WGS)	McHugh et al. (2020)	Psychrotrophs and thermophiles enriched	HTS used to track species across chain
Farm to milk powder	Culture-dependent	Paludetti et al. (2019)	Microbial quality better at mid lactation than late	Management practices influence milk quality
Farmstead cheesemaking	Multiple sample types; 16S and ITS	Sun and D'Amico (2021)	Transfer along the farmstead cheesemaking continuum (bidirectional)	Important role of traditional processes for cheese ripening microbes

biofilms in the dairy animal housing, including dairy (Guéneau et al., 2022).

There were few research studies (n = 5) across farm and processing facilities (whether on farm or separate artisanal facilities; Table 1), all conducted within the past 6 years. Farmstead combined with processing was addressed by Cruz-Facundo et al. (2023), Gajewska et al. (2022), McHugh et al. (2020), Paludetti et al. (2019) and Sun and D'Amico (2021).

On-farm studies (n = 8) focused on farms and small dairies (Rios-Muñiz et al., 2019; Kamimura et al., 2020; Liu et al., 2024), dairy housing and teat preparation (Doyle et al., 2017a; Du et al., 2020), fecal contamination of waterways (Devane et al., 2023), cow drinking water (Hayer et al., 2022), and well water (Xue et al., 2020). Also related to the farm environment, milk collection was addressed in 5 studies (Table 2; Lindell et al., 2018; Du et al., 2020; Latorre et al., 2020; Pacha et al., 2021; Porcellato et al., 2021). Storage and transport of milk were the focus of 2 studies (Pantoja et al., 2009; Ban et al., 2023) (Table 3).

A total of 14 studies pertained to the dairy processing facility only (Table 3, Table 4), including raw or pasteurized milk (Malek et al., 2012; Elegbeleye and Buys, 2020; Du et al., 2022), dairy facilities (Kocurek et al., 2023), artisan or traditional cheese (Bokulich and Mills, 2013; Carpino et al., 2017; Gaglio et al., 2019; Castro et al., 2020), cheese facilities (Dzieciol et al., 2016; Schön et al., 2016; Johnson et al., 2021; McHugh et al., 2021; Lacorte et al., 2022), and 1 for milk powder facilities (Wang et al., 2018).

Interest in microbial communities of biofilms in the production and processing environment has a wide range of publication dates between 2008 and 2024. The methods for analyzing these communities have greatly evolved over the period from mostly culture-dependent (n = 10; Vlková et al., 2008; Pantoja et al., 2009) to mostly culture-independent (n = 14), with additional studies combining both (n = 6). As the main molecular methods used to analyze dairy biofilms have been reviewed (Yuan et al., 2023), only a brief description is provided here to distinguish among the studies included. The molecular methods can essentially be classified as either targeted (to specific genes, such as 16S rRNA, or virulence genes, such as quantitative PCR [**qPCR**]) or untargeted (shotgun or whole genome sequencing). Among the older culture-independent techniques of molecular microbial ecology, denaturing gradient gel electrophoresis (**DGGE**) is a targeted technique based on separating 16S rRNA gene amplicons of equal size by their GC content on a chemical denaturing gel (Rasolofo et al., 2011). This method provides bands that can then be cloned and sequenced for validating the identity of the species. One study relied on DGGE with verification of clone sequences to support the species attribution (Carpino et al., 2017). That study was able to show the contribution to in cheese typicity of volatiles produced by bacterial biofilms on wooden vats. As sequencing became high-throughput and affordable, targeted metagenetic sequencing of 16S rRNA gene amplicons was developed, where sequences are acquired from a library of amplicons obtained from PCR amplification of a microbial community DNA sample (Meola et al., 2019). This technique was used on its own in 8 studies (Doyle et al., 2017b; Wang et al., 2018; Du et al., 2020; Kamimura et al., 2020; Porcellato et al., 2021; Lacorte et al., 2022; Ban et al., 2023; Devane et al., 2023), with fungal internal transcribed spacer (**ITS**) amplicons added in one study (Sun and D'Amico, 2021). With further lowering of sequencing costs, untargeted sequencing of all the DNA in a sample could be carried out by various methods of fragmenting the DNA and attaching adapters for library preparation for whole genome sequencing (**WGS**) or shotgun sequencing (Xu et al., 2023). Six studies combined multiple molecular techniques, including 16S rRNA gene and 18S rRNA (eukaryotic microbes) or ITS amplicon sequencing (fungi), qPCR, or WGS (Bokulich and Mills, 2013; Dzieciol et al., 2016; Schön et al., 2016; McHugh et al., 2020, 2021; Kocurek et al., 2023).

DISCUSSION

The utility of a scoping review is first to contextualize the current literature with respect to past reviews that have identified research gaps, and next to provide recommendations for future research, to avoid duplication and focus in on the converging opinions.

Flint et al. (2020) reviewed over 20 years of progress on biofouling in dairy production and processing with both historical and analytical perspectives, dividing up the dairy manufacturing plant into problem areas according to the conditions favoring the growth of typical microbial groups in each type of processing equipment (temperature, water, nutrients). The farm environment is mentioned as a source of contamination for specific pathogens, and farm conditions have been simulated in a model as a research tool for determining the effectiveness of clean-in-place (**CIP**) systems against bacterial spores (Ostrov et al., 2016). The risk of residual contamination is higher when biofilms shield pathogens and contaminants, which are harder to remove, thus reducing the effectiveness of the cleaning process. This analytical review identified the limitations in knowledge of bacterial interactions and the impact of inadequate cleaning on perpetuating biofilm contamination, suggesting the need for improved evaluation of the effectiveness of cleaning procedures (Flint et al., 2020).

Milk spoilage agents and pathogens readily contaminate milking equipment throughout the dairy farm and

Table 2. Extracted peer-reviewed studies related to tracking biofilms in the dairy production and processing environments: production and milk collection environments (n = 13)

Location and product	Microorganism and methods	Reference	Highlights of the study	Conclusion
Production				
Feces; streams	Microbiota (16S)	Devane et al. (2023)	Indicators of fecal pollution to waterways	Mobilization from cowpats after rainfall
Housing and teat preparation	Raw milk microbiota (HTS)	Doyle et al. (2017a)	Influence of farm practices on raw milk microbiota	Environment is the main driver of milk microbiota
Housing	Microbiota (16S)	Du et al. (2020)	Variability between 2 farms	Transfer of contamination from feces to milk
Drinking water	Culture-dependent and ATP tests	Hayer et al. (2022)	Impact of biofilms on water quality	Biofilms in troughs are reservoirs of AMR[1] bacteria
Artisanal farms	Microbiota (16S) Multiple sample types	Kamimura et al. (2020)	Variability of microbiota among farms	Starter bacteria shape the microbiota of final product
Dairy farms	*Cronobacter* spp. (culture-dependent)	Liu et al. (2024)	50% of 40 isolates produced strong biofilm	Genetic similarity of isolates suggests cross-contamination
Small dairies	Culture-dependent (multiple types)	Rios-Muñiz et al. (2019)	Longitudinal analysis over 2 yr; biofilm formation tested in vitro	Raw milk did not meet safety and quality standards
Well water	Pathogens (culture-dependent and independent)	Xue et al. (2020)	Final meters of water hoses at risk for biofilms	Biofilms serve as food for amoebas
Milk collection				
Milking	Microbiota (16S)	Du et al. (2020)	Teat liner and teat dip cup	Focus on disinfection and cleaning
Combined surfaces in contact with milk	*Staphylococcus aureus* (culture)	Latorre et al. (2020)	Scanning electron microscopy showed biofilms on milking equipment	Rubber liners had similar isolates to infected cows
Rubber liners and tubes	ATP bioluminescence	Lindell et al. (2018)	Variation in cleaning efficiency	Hygiene quality maintained over lifetime of rubber parts
Bulk tank milk (BTM) and surfaces	*S. aureus* culture-dependent	Pacha et al. (2021)	Visible adherences in milking equipment contain similar isolates to BTM	Residues in the milking system contaminate BTM
BTM (n = 37 farms)	Culture-independent (16S)	Porcellato et al. (2021)	Longitudinal sampling (7 mo)	Weather and feeding had greater impact

[1]AMR = antimicrobial resistant.

Table 3. Extracted peer-reviewed studies related to tracking biofilms in the dairy production and processing environments: studies covering storage, transport, and processing of milk (n = 14)[1]

Location and product	Microorganism and methods	Reference	Highlights of the study	Conclusion
Storage and transport				
Raw milk	Microbiota (16S)	Ban et al. (2023)	Dominant taxa at 4°C and 25°C after 7 d	Diversity decreases with storage time
Raw bulk milk	Culture-dependent (coliform counts, SCC, TBC, LPC)	Pantoja et al. (2009)	Increased coliform counts with higher SCC and milk temperature	Variability among 16 farms; no correlation of LPC to other indicators
Processing				
Artisan cheese	Microbiota (HTS, qPCR)	Bokulich and Mills (2013)	Processing area drives diversity more than facility	Importance of production environment as source of fermentation microbes
Cheese facilities (n = 10)	Microbiota (DGGE)	Carpino et al. (2017)	Biofilms of wooden vats add to volatiles in cheese	Role of biofilms in cheese typicity
Artisan cheese	*Staphylococcus aureus*	Castro et al. (2020)	82% of isolates biofilm positive on polystyrene	Biofilm was the main virulence factor
Pasteurized milk	Culture-dependent; 16S	Du et al. (2022)	Food safety standards met, but contaminants from equipment and air	Quality control sampling needed during pasteurization process
Raw, pasteurized, ESL milk	*Bacillus subtilis* and *Bacillus velezensis*	Elegbeleye and Buys (2020)	Most isolates formed biofilms; thermophiles from soil found in products	Interventions needed to prevent adhesion in processing plant
Cheese facility (wood)	Microbiota culture-dependent and independent	Gaglio et al. (2019)	Significant correlation of specific VOC and *Lactococcus* OTU	Wooden vats do not compromise the safety of Sicilian cheese
Cheese facilities (n = 3)	Microbiota (16S and culture)	Johnson et al. (2021)	Daily changes in microbiota	Facility differences in microbiota due to starter
Dairy facilities (swabs)	*Listeria monocytogenes* (culture, 16S, and WGS)	Kocurek et al. (2023)	Only 3× genome coverage needed to detect *L. monocytogenes* after culture enrichment	Level of coverage needed to source track pathogens
Cheese facilities (n = 6)	Microbiota (16S)	Lacorte et al. (2022)	Deficient food safety management system (FSMS) related to higher diversity among hands, surfaces, and product	Quality of FSMS important
Pasteurized milk facilities (n = 5)	*Bacillus cereus* (culture)	Malek et al. (2012)	Resistance of *B. cereus* biofilm to quaternary ammonium disinfectant	CIP ineffective in eradicating biofilms on equipment
Processing facility	Microbiota (16S and WGS)	McHugh et al. (2021)	Low DNA yield from swabs is challenging	Great potential, but problems with methods
Milk powder facilities	Microbiota (16S PacBio)	Wang et al. (2018)	Milk storage led to higher contamination of final product	Tracking can identify contamination source

[1]ESL = extended shelf life; TBC = total bacterial count; LPC = laboratory pasteurization count; HTS = high-throughput sequencing; VOC = volatile organic compounds.

Table 4. Extracted peer-reviewed studies related to tracking biofilms in the dairy production and processing environments: studies covering floor drains in dairy processing facilities (n = 2)

Location and product	Microorganism and methods	Reference	Highlights of the study	Conclusion
Floor drains				
Cheese facility (n = 1)	Microbiota (16S, 18S, qPCR)	Schön et al. (2016)	Product drives the profile of drain biofilms with low diversity	Importance of sampling drain biofilms in addition to drain water
Cheese facility (n = 1)	Biofilm and *Listeria* (16S, qPCR, culture)	Dzieciol et al. (2016)	*Listeria* at the limit of detection without enrichment	Importance of sampling drain biofilms in addition to drain water

processing environment. This contamination can be further exacerbated by biofilm formation within the equipment, potentially leading to resistance against cleaning procedures. Marchand et al. (2012) explored this concern, evaluating the effectiveness of cleaning agents and CIP procedures along with reviewing methods for biofilm monitoring and detection. Marchand et al. (2012) suggest a possible link between biofilm formation within milking machines and their increased resistance to disinfection procedures. Multispecies biofilms give evidence of the cooperation of spoilage and pathogenic biofilm-forming bacteria. This review highlights the need for better knowledge of persistent sources of contamination, with a system for measuring cleaning efficiency (similar to the emphasis by Vlková et al., 2008 and Flint et al., 2020). Marchand et al. (2012) also emphasized the need for coating strategies and fortified sanitizers, with the aim of improving cleaning procedures. As cited in Machado et al. 2017), Jindal et al. (2016) compared 4 stainless steel coatings, showing that viable counts of 4 milk spoilage spore-formers were lower on stainless steel coupons coated with Ni-P-PTFE than on untreated stainless steel.

Machado et al. (2017) reviewed sources of contamination of raw milk, particularly focusing on psychrotrophs and heat-stable enzymes in spoilage. Biofilm formation in milking equipment and storage tanks can significantly increase the microbial load of milk, highlighting the importance of proper cleaning and disinfection (**C&D**) practices. Therefore, implementing screening procedures at the dairy processing plant could be used to direct the milk to an appropriate processing stream based on its microbial load, potentially separating milk with a higher contamination risk. This approach could be difficult to implement without real-time rapid testing methods.

Psychrotrophic bacteria and their heat-stable enzymes are a major concern in the dairy industry, causing significant economic losses due to product spoilage throughout the world (Yuan et al., 2019). In a more recent review, Yuan et al. (2019) highlighted the importance of this issue by focusing on heat-stable enzymes produced by psychrotrophic bacteria. These authors suggest that reliable prediction methods could help to reduce contamination of raw milk from milking to processing. Pathogens have been the subject of recent reviews, with respect to antibiofilm intervention strategies and antimicrobial resistance in biofilms (Butucel et al., 2022).

Two recent reviews focus on multiomics applied to the dairy sector (Joshi et al., 2022) and to biofilms in particular (Yuan et al., 2023). Joshi et al. (2022) conducted a semisystematic review showing that conventional microbial risk assessments generally overlook factors such as biofilm formation. They advocate for incorporating omics technology into quantitative risk assessment models and provide an overview of recent quantitative microbial risk

assessment (**QMRA**) applications to dairy foods. This approach could be significantly enhanced by a deeper understanding of biofilm formation, as explored by Yuan et al. (2023). Their review summarizes the use of multiomics tools for studying biofilms in the dairy industry. Both reviews highlight the need for future development of adequate statistical models to analyze omics data in the context of food safety. Additionally, addressing data heterogeneity and improving data analysis reliability are essential for maximizing the value of omics technologies (Joshi et al., 2022; Yuan et al., 2023).

Progress in dairy sanitation over the past 100 years was reviewed by Rankin et al. (2017), who provided a historical perspective, but also a farsighted glimpse into the future of the improvement of CIP equipment. At that time, 2 main challenges were recognized: documenting processing parameters, including measures for compliance and second, reducing the footprint of milk processing (water treatment, processing and recovery, heat transfer, novel designs, to name a few). This review reminds us of changes in processing, such as extended run times and the use of novel heat exchange devices, that can create unforeseen problems with biofilm formation. Another example is the study of the control of biofilm formation on filtration membranes to reduce food spoilage due to spore-forming bacteria (Anand et al., 2014). The corollary is that risk of biofilm formation should become an integral part of process control.

The positive benefits of biofilms in livestock buildings represent a novel, emerging aspect of biofilm management (Guéneau et al., 2022). Beneficial bacteria could embody an innovative biosecurity enhancement strategy in housing, as biopreservation and positive biofilms are currently used in other agricultural settings, such as poultry litter (Guéneau et al., 2022). This approach leverages the natural competitive advantage of specific microorganisms applied by spraying surfaces in livestock buildings before colonization by undesirable bacteria that enter through organic matter from feed and feces. By establishing themselves on surfaces that have been cleaned and disinfected, these beneficial bacteria could prevent colonization and outcompete undesirable bacteria, including pathogens, through various mechanisms, such as the production of antimicrobial or antiadhesive compounds, competitive exclusion, or nutritional competition. The advantages of such an approach could reduce the reliance on harsh chemical disinfectants and concomitant problems, leading to a greener approach to biosecurity in livestock buildings. In addition to excluding pathogens on surfaces, positive biofilms could have a wider impact on animal health (as modulators of the microbiota of the digestive tract) and circulating beneficial bacteria in the food supply chain (Guéneau et al., 2022).

With these reviews in hand, the synthesis of the remaining 34 studies will focus on identifying converging viewpoints and potential avenues of investigation.

Agricultural Practices and Biofilm Formation

As evidence from the recent reviews, farm studies have primarily been interested in biofilm formation in the context of animal health for controlling the risk of transmission of mastitis and other diseases through the milking equipment (Butucel et al., 2022), and then to milk consumed by humans. Butucel et al. (2022) recently reviewed farm biosecurity measures and practices, specifically with respect to biofilms in the farm environment, mainly from the perspective of the risks for pathogen transmission and the amplification of antimicrobial resistant microbes. Antimicrobial and antibiofilm intervention strategies are also reviewed for several livestock operations, including dairy (Butucel et al., 2022). Essentially, after stating the currently applied intervention methods, the prospects from this review make an appeal for more research on how to reduce bacterial biofilms through improved farm management practices. This focus aims to minimize the development of resistance and tolerance to biocides and novel antimicrobials.

Biofilms in milking equipment surfaces could be a source of *S. aureus*, even after cleaning and sanitation procedures. These biofilms are a significant source of *S. aureus* contamination for both bulk tank milk and cows (Latorre et al., 2020). Samples were collected from milking equipment pieces that exhibited visible macroscopic adherences. Scanning electron microscopy confirmed the presence and characteristics of biofilms on these surfaces. Milk collected during late lactation and winter months tends to have higher microbial loads (Paludetti et al., 2019). This study also noted that late-lactation milk had lower microbiological quality compared with mid-lactation milk. Transport conditions and cleaning protocols were cited as possible determinant factors influencing the microbial load of milk.

Studies designed with short-term sampling (1 or 2 sampling times) provide fixed views delimited by time that may denote sporadic events. In contrast, longitudinal sampling conducted over months or seasons may lead to divergent views on the stability and variation of the microbiota in the farm environment. For example, short-term shifts in the microbiota of bulk tank milk over weeks during a 7-mo period were shown to be related to mastitis (*Staphylococcus* and *Streptococcus*) and the influence of season (Porcellato et al., 2021). However, when the same group reanalyzed milk from the same 37 farms in Norway a year later, a different picture emerged. Long-term shifts in the microbiota seemed to be related to weather and feeding practices, not the type of milking equipment used

(Porcellato et al., 2021). More spoilage bacteria were detected in 2019, when the average rainfall was higher than in 2017. A major difference between the 2 sample years was the weather during the harvest season. The previous summer's dry temperature reduced crop production, necessitating an increase in the amount of concentrate and purchased imported roughage in the feed in 2019. The advantages of this study design allowed the authors to minimize the sources of variation due to season, hygiene practices, milk storage, and sampling routines in order to associate specific events and recorded factors to changes in the microbial profile. Documentation of the specific farm context is thus key to cause-and-effect attribution to the seemingly stochastic changes in microbial profile, such as blockage in the ball sprayer for the bulk tank, filter sock management, cooling, and CIP parameters such as water temperature and pressure. Over the 2 years, several farms switched from a parlor milking system to an automatic milking system (**AMS**), thus raising the possibility that less efficient teat cleaning associated with AMS could be driving an increase in bacterial diversity (Porcellato et al., 2021). Further investigation would be necessary to establish a causal link between milk contamination levels and biofilm development among multiple configurations of AMS.

During milk transportation, several factors may influence biofilm development on the interior surfaces of tankers, such as the frequency and efficacy of truck sanitation and distance traveled to collect milk from farms and deliver it to the processing plant. In practice, it has been shown that tanker hauling over 24 h of continual use and cleaning practices both have little effect on the milk microbiota (Darchuk et al., 2015a,b). Although biofilm formation was not studied, minimal biofilm formation has been suggested, due to the cold temperature, low shear, and smooth surface area in comparison to processing equipment materials (Darchuk et al., 2015a). The authors propose that variation in the efficiency of tanker sanitation would only cause sporadic issues in the downstream processing that would be difficult to trace (Darchuk et al., 2015a,b). The authors suggest that the focus for sanitation should be on the post-haul CIP practices rather than between-load rinse and sanitizer treatments (Darchuk et al., 2015b).

In a large-scale study of 899 tanker trucks over 3 seasons, the microbial community structure of raw milk from tanker trucks in California was highly variable (Kable et al., 2016). More than 50% of the taxa were present at low abundance (under 1%). However, a core microbiota present in 100% of the samples was identified, consisting of the 5 major phyla: *Bacillota* (synonym *Firmicutes*), *Actinomycetota* (synonym *Actinobacteria*), *Bacteroidota* (synonym *Bacteroidetes*), *Pseudomonadota* (synonym *Proteobacteria*), and *Mycoplasmatota* (synonym *Tenericutes*; a total of 18 families). Interestingly, the study found that *Pseudomonas* was not part of this core microbiota, suggesting that the amplification of this group of psychrotrophic bacteria occurs during processing, but that its occurrence during transport is sporadic in this case. One of the factors influencing this aspect would be the efficiency of cooling (Elmoslemany et al., 2009, 2010) or the residence time in the bulk tank before the milk is collected. When milk production is down, milk collection may be less frequent, leading to longer holding times. Changes in core raw milk microbiota with region reflect the complex interplay between farm management practices and milk microbiota (Ouamba et al., 2022).

On-farm processing provides special advantages for potentially minimizing holding and transport time, unless the daily production is insufficient, thereby requiring the accumulation of enough milk for the processing capacity. Increasing bulk tank storage time would generally favor an increase in psychrotrophic load, given the growth rate of psychrotrophic bacteria (Elmoslemany et al., 2009). The farm environment also plays a role in shaping the cheese microbiota. In a study by Falardeau et al. (2019), a significant increase in *Bacillota* (synonym *Firmicutes*) was observed, from 31% on farm to 92% in the final cheeses (Cheddar, Gruyère, Jarlsberg, and Brie), where the cheesemaking plant was located at a distance of 25 km from the farm. A total of 32 out of 37 operational taxonomic units (**OTU**) found in the cheese were also present in the farm samples. As expected, a higher abundance of environmental *Pseudomonadota* (synonym *Proteobacteria*) was found in cheeses made with raw milk compared with cheeses made with heat-treated milk. As the methods did not involve sanitizing or disinfecting before swabbing, it can be assumed that the samples represent the soiled state, not the biofilms remaining after cleaning.

Two recent studies targeting small on-farm cheese facilities focused on 2 foodborne pathogens, namely *Bacillus cereus* (Cruz-Facundo et al., 2023) and *S. aureus* (Gajewska, et al., 2022). Both studies employed culture-dependent methods to isolate bacteria and then used in vitro screening for pellicle or biofilm formation. This test showed that many or most of the isolates were biofilm-formers (87.5% for *B. cereus* and 62% for *S. aureus*). Of importance, the *B. cereus* cheese isolates were closely similar to the airborne isolates (to the limits of the genotyping methods employed; Cruz-Facundo et al., 2023). Unsurprisingly, raw milk cheeses were deemed at risk for *S. aureus* (Gajewska, et al., 2022).

Three additional studies covering the farm to processing continuum encompassed milk powder processing (n = 2; Paludetti et al., 2019; McHugh et al., 2020) and cheesemaking (Sun and D'Amico, 2021), which will be discussed in the next section.

Biofilm Formation in Dairy Processing Environments

Cross-facility studies provide a unique opportunity to connect the farm microbiota with that of the processing plant, distinguishing the impact of processing from that of milk collection and transport. McHugh et al. (2020) conducted a study in Ireland using both 16S rRNA gene amplicon sequencing and shotgun sequencing to track the microbial profile of milk from 67 farm bulk tanks to skim milk powder over 2 periods representing mid and late lactation. They collected samples from 11 collection tankers, 2 whole milk silos, 2 skim milk silos, and 1 cream silo, finishing with 3 triplicate samples of skim milk powder. The data revealed a transformation of the dairy microbiome as it progressed through the manufacturing process. Psychrotrophic spoilage bacteria increased over storage time in the raw milk silo, with a dominance of *Pseudomonas*, *Acinetobacter*, and even the *Lactococcus* genus (McHugh et al., 2020). Skim milk powder produced from mid-lactation showed an enrichment of thermophilic spore-formers. In contrast, spoilage psychrotrophs dominated the skim milk powder from late-lactation milk. Paludetti et al. (2019) conducted a concurrent study using culture-dependent methods. Their study, which compared mid-lactation bulk tank milk (n = 67) to late lactation (n = 150), also found that skim milk powder produced with late-lactation milk was lower in quality (Paludetti et al., 2019). Although neither study was designed to examine biofilms, both concluded that biofilm formation was probably the cause of higher levels of contaminating thermophilic or spore-forming bacteria.

The duration and temperature of cold storage drive the switch from a predominance of gram-positive microorganisms in fresh milk to a predominance of gram-negative bacteria. Even under hygienic conditions practiced at milking, a low psychrotroph count equivalent to 10% of the total mesophilic aerobic count in freshly collected milk can reach 90% of the total count after reception at the processing plant (Machado et al., 2017). Numerous studies have established the shift in microbiota of raw milk in the silo at the processing facility without necessarily testing for biofilm presence on the equipment (Kable et al., 2016). The distinct community structure of milk among silos (n = 5) implied that persistent biofilms could be added to the determining factors along with cold storage and season (Kable et al. 2016). Despite these variations, a core milk microbiome endures. Kable et al. (2016) noted that endospore-forming bacteria, including *Bacillus* and *Clostridium*, are prevalent members of this core. These taxa are commonly found on dairy farms and known to thrive in dairy processing environments. Notably, species within these genera are associated with spoilage of pasteurized milk and milk products.

Heat treatments in dairy industries do not eliminate bacterial spores found in milk, which can attach to stainless steel surfaces and form a biofilm. The relationship between spores and biofilms is cyclical, with each ensuring the survival of the other. Despite technological advances, spore-formers are a major contaminant of heat-treated milk and cause problems with shelf life. In the dairy industry, the adhesion of thermophilic *Bacillus* spores to stainless steel increases 10 to 100 times in the presence of skim milk. Control measures include shortening production cycles, increasing cleaning frequency, using disinfectants, and reducing the surface area in optimal temperature zones (Malek, 2019). The findings highlight the complex interplay between raw milk microbiota, processing environments, and the persistence challenges posed by spore-forming bacteria and biofilms. Further research is needed to fully understand the factors influencing microbial shifts in silos and to develop more effective strategies to mitigate the risks associated with spore-forming bacteria in dairy products.

The sporadic or intermittent nature of contamination events in the dairy environment can obscure overall trends in the resident microbiota of processing facilities. Short-term studies can be contrasted with longitudinal studies to delineate some of the sources of variability. Johnson et al. (2021) examined 3 Cheddar cheese production facilities over 3 d of production. In this continuous process, they were able to take repeated samples of the processing equipment over 12 min for each day. They combined 16S rRNA gene amplicon sequencing with culture-dependent plating, revealing variability over time and space, among facilities, and even between days within the same facility. This study was able to identify sequence variants (amplicon sequence variant or sequence variant) explaining at least 5% of the variance over the 2 main components of the principal component analysis. As the facility surfaces were sampled without cleaning, the authors were able to show the build-up of nonstarter bacteria on belts (draining-matting conveyor [**DMC**]) over the production day. This study underscores the importance of subdominant members of the microbial community that contribute later in the ripening step, which may be masked by the dominant starter bacteria. The variation between facilities was attributed first to the pasture-based milk source of facility A, in contrast to the common milk source for facilities B and C. Given the same milk, the variation in microbiota was unexpected, so the thermization process and age of the equipment in each facility were added as possible explanations for the variance. In particular, the equipment in facility A was older, leading to accumulation on DMC belts of species associated with biofilm formation. Indeed, the older age of the DMC at facility A in operation for ~10 to 15 years longer than those at facilities B and C, may have led to increased biofilm formation and

accumulation of biofilm-associated bacteria. This could explain the higher bacterial load and species richness observed on the DMC belts at facility A. Within each facility, production days also differed in microbial profile. Extended sampling across multiple production cycles is needed to overcome the limitation of short-term studies, as employed by Johnson et al. (2021). Daily variations in microbial profiles have been observed even when facilities use identical processing equipment and milk sources. These variations could be attributed to factors such as the rotation of starter cultures and differences in equipment age or design, potentially influencing biofilm formation. Biofilms, in turn, can harbor diverse microbial communities and contribute to the overall complexity of the cheese microbiome (Bokulich and Mills, 2013).

This additional source of temporal variation can obscure the identification of an in-house microbiota typical of each facility, a concept previously proposed (Bokulich and Mills, 2013). Reducing the diversity of milk microbiota through the processes of pasteurization and cleaning, as well as the use of defined starter cultures, has raised a concern for cheese distinctiveness or typicity (Sun and D'Amico, 2021). Although starter cultures ensure consistent product quality and safety, they can potentially homogenize cheese flavor profiles. This contrasts with traditional cheesemaking practices, where the resident microbiota of the processing environment played a substantial role in shaping the final product characteristics. Traditionally, the processing environment played a substantial role in shaping cheese characteristics through the resident microbiota. A study on traditional farmstead cheese production process (Bethlehem cheese) supports this concept (Sun and D'Amico, 2021). This study revealed the role of the processing environment, particularly wooden vats, in harboring and transferring diverse microbial communities to the cheese (Gaglio et al., 2019). This emphasizes the concept of biofilms on wooden surfaces within the cheesemaking environment. These biofilms act as reservoirs of diverse microbes, including lactic acid bacteria and fungi, that can be transferred to the milk during processing. This is in agreement with a study of farmstead and artisanal cheese products that has demonstrated the value of traditional cheesemaking tools and processes to the typicity of cheese, as well as its safety (Gaglio et al., 2019). Biofilm composition is influenced by factors such as the repeated use of these tools and the presence of raw milk. Interestingly, the exchange appears to be bidirectional, as the transfer of cheese ripening bacteria such as *Brevibacterium* from rinds to wooden boards and back has been highlighted by Sun and D'Amico (2021), contributing to the development of typical rind color and texture. This continuous exchange between the cheese and its surroundings highlights the dynamic nature of the cheese microbiome and the intricate role played by the processing environment in shaping cheese characteristics.

Dairy food contact surfaces were surveyed after C&D by Maes et al., (2019), who found that 87% of isolates obtained from surfaces after C&D exhibited some spoilage potential. The dominant taxa still adhered after cleaning included *Pseudomonas*, *Microbacterium*, *Stenotrophomonas*, *Staphylococcus*, and *Streptococcus*. Even though the next study was done in vitro, Sadiq et al. (2024) were able to demonstrate that the reaction of multispecies biofilms to C&D are not the sum of the pairwise interactions, suggesting that the biofilm persistence will depend not only on the presence of individual species, but also on the initial colonizing combination.

The microbiota of floor drains was shown to vary depending on the site in the processing facility, with little overlap between the drain water and drain biofilm (Dzieciol et al., 2016). This study identified a diverse range of bacteria in floor drains, including *Pseudomonas*, *Leuconostoc*, *Lactococcus*, and *Janthinobacterium*. Notably, the composition of these bacterial communities directly reflected the processing activities in different areas. The drain biofilms in the cooling area, cutting area, washing area, and processing area were correlated with the products manipulated in those areas, such as *Lactococcus lactis* from fresh cheese in the drain of the area where cheeses were cooled (Dzieciol et al., 2016). As expected, *Pseudomonas* was more prevalent in drain biofilm compared with drain water. This study reinforces the importance of including sampling of drain biofilms, in addition to drain water, in *Listeria monocytogenes* monitoring. By analyzing both sample types, a more comprehensive picture of the microbial community present can be obtained, potentially increasing the chance of detecting *L. monocytogenes* and improving food safety practices (Dzieciol et al., 2016).

Schön et al. (2016) analyzed the microbial communities in floor drains of an Austrian cheese production facility. They found that bacteria and yeast associated with soft and semi-hard cheesemaking, such as *Lactobacillus* and *Debaryomyces*, dominated the drain microbiota. The authors also observed a low level of water-associated taxa. The relatively low diversity was attributed to the use of chlorine disinfectants. Again, Schön et al. (2016) reported only moderate overlap between drain water and drain biofilm communities with the drain water, underscoring the importance of collecting biofilm samples for a more complete picture of the drain microbiota.

Emerging Methods and Technologies: Limitations of Past Methods

Biofilms formed by pathogenic or spoilage microorganisms have become serious issues in the dairy industry,

as this mode of life renders such microorganisms highly resistant to CIP procedures, disinfectants, desiccation, and other control strategies. The advent of omics techniques, particularly the integration of different omics approaches (e.g., genomics, transcriptomics, proteomics, metabolomics), has greatly improved our understanding of the features of microbial biofilms. By analyzing various biological molecules (genes, RNA, proteins, and metabolites), omics techniques provide a more comprehensive picture of the complex processes occurring within biofilms.

The recent review by Yuan et al. (2023) has summarized the studies applying multiple omics methods to the analysis of dairy biofilms, mostly from in vitro investigations published between 2019 and 2022. Challenges were identified concerning experimental design, and a gap was noted in simulating the dairy-associated environment (temperature, flow, pH, contact material, and nutrients). Yuan et al. (2023) also highlight the lack of standards in analysis of omics data (for example, data cleaning, transformation, and normalization, as well as data management, including archiving and sharing), stressing the need for reproducibility and facilitating data integration in further research. The development of machine learning tools was suggested as a means of facilitating the prediction of pathways of biofilm formation.

Joshi et al. (2022) conducted a semisystematic review exploring the potential of multiomics for QMRA in the dairy sector. Although this application is specific to pathogens, it does have potential for spoilage agents and biofilm formation in dairy processes. However, the authors highlighted a key limitation: genotypic variations identified through omics are not directly associated with the phenotypic behavior of microbial populations, thus suggesting a limitation to the interpretation of omics results, but still supporting the usefulness of the approaches.

Culture-based analyses introduce somewhat of a selection bias depending on the media used, and whether isolate selection was randomized or arbitrary. Counts on M17 medium were shown not to be specific to lactic acid bacteria, because the authors obtained numerous isolates of *Kocuria* spp. for example (Gagnon et al., 2020). Nevertheless, for practical applications, plate counts of general groups of microbes such as the laboratory pasteurization count and *Enterobacteriaceae* are more useful in diagnostics of unhygienic conditions than knowing the exact species composition (Martin et al., 2018).

Sample preservation has been the subject of much debate (McHugh et al., 2021). As a gold standard, freezing with a cryoprotectant such as dimethyl sulfoxide (**DMSO**) provides a measure of microbial community stability during sample transit and storage (Ouamba et al., 2020). Ouamba et al. (2020) showed that combining azidiol with DMSO provided an optimal preservation method for maintaining viable cells, whether at −20°C or for 10 d at 4°C. Converging opinions on DNA extraction from dairy samples emerge from this current review, due to the low biomass of raw milk and environmental swabs from equipment after cleaning. Ganda et al. (2021) showed that magnet-based methods of DNA extraction are superior for isolating DNA from bacteria in bovine milk. This study recommended that more efforts be made to standardize protocols for DNA extraction from low-biomass samples such as raw milk (Ganda et al., 2021). McHugh et al. (2021) also highlight the issue of DNA extraction from samples with low microbial load, which might limit the sequencing technology that can be used. Two sequencing technologies have been compared, MinION (Oxford Nanopore Technologies) and Illumina NextSeq, revealing comparable species level classification. The main limitation of this approach for routine monitoring of microbial communities is the high quantity and quality of DNA required (McHugh et al., 2021).

Some limitations to analyzing sequence data become apparent from this dataset, with respect to core microbiota versus temporal and spatial variability. When searching for core microbiota, many studies either filter out or pool all reads below 1% in a category called “others,” as well as filtering for taxa appearing in 90% to 100% of samples. As a corollary to this, the subdominant taxa under 1% represent the distinctive features of each sample, which do not occur in the majority of samples. Another limitation of the 16S rRNA gene sequencing method, in addition to representing all DNA of cells (whether viable or not), is that pathogens are generally present at very low levels, requiring enrichment. Multivariate statistics can be useful to address this level of complexity using the sequence reads, rather than the diversity indices. The purpose of ecological diversity measures is to describe the number and distribution of taxa as a whole among samples (homogeneous or heterogeneous), not to determine the similarity of taxa profiles. Thus, analysis of sequence data representing dairy environments and products should include a clear strategy for describing the sample variability, consisting of complementary approaches that recognize both the core and the distinctive members of the taxa. For example, statistical methods can work with compilations of amplicon sequence variants (ASV) instead of taxa (OTU), giving a finer resolution of changes in the microbial profile. This concept has been comprehensively reviewed by Callahan et al. (2017). Although nonmetric multidimensional scaling of abundance measures portrays sample separation with dimension reduction, partial least squares discriminant analysis provides variable importance scores that highlight the significant sequences contributing to the classification labels (Marcos-Zambrano et al., 2023). This essentially contrasts unsupervised (exploratory) with

supervised (predictive) statistical methods, where supervised approaches use labeled datasets to train algorithms to predict outcomes, thus determining the contribution of the classification factors to explaining the variation among the samples (Marcos-Zambrano, et al., 2023). Johnson, et al. (2021) defined the "others" category as the proportion of sequence variants that contributed less than 5% of the variance, a solution that ranks the groups of sequences according to their importance.

Risk Management

The One Biofilm concept, similar to the One Health concept, emphasizes the interconnectedness of biofilm formation in humans, animals (domestic and wild), and the environment (Jacques and Malouin, 2022). The One Biofilm concept, introduced by Jacques and Malouin (2022, p. 51), emphasizes the importance of a "collaborative, multi-stakeholder, multisectoral and transdisciplinary approach to address complex problems involving biofilms." This concept aligns perfectly with the growing focus on risk management in the dairy industry. Biofilms can harbor and protect pathogenic and spoilage microorganisms, making them a critical factor in risk assessment. The goal of controlling biofilm formation would be the prevention of the persistence and spread, particularly of pathogens, across environments, animals and humans. In view of sustainability, adding spoilage agents to this would extend the concept to reducing food waste by improving food quality and shelf life, as well as food safety.

Quantitative microbial risk assessment, along with the One Biofilm concept, plays an essential role in improving food safety in the dairy industry. Quantitative microbial risk assessment provides a quantitative assessment of the risk of foodborne illness, taking into account the formation of biofilms throughout the dairy production chain (Ramos et al., 2021). The integration of QMRA with the One Biofilm concept provides a more comprehensive approach to risk management. This combined approach makes it possible to identify critical control points where biofilms are likely to form and present an increased risk of contamination, as well as assess the impact of biofilm formation on risk at each stage of production.

Omics provide valuable information on the composition and functioning of microbial communities in biofilms, enabling the identification of microorganisms responsible for degradation and the targeting of more effective interventions to control them (Joshi et al., 2022). In summary, the One Biofilm concept, QMRA, and omics analysis constitute a powerful toolbox for the dairy industry. By integrating these elements, the dairy industry can adopt an approach to mitigating the risks associated with biofilms, thereby ensuring the safety and quality of dairy products.

This review has attempted to convey the limitations of study design and selecting which factors to focus on, such as the influence of lactation period and environmental factors, on the microbiological quality of milk and subsequent skim milk powder (**SMP**). For example, Paludetti et al. (2019) highlighted that the study design did not allow for statistical validation of the hypothesis that stage of lactation or environmental factors, or both, related to the time of year influence microbiological quality. Additionally, the study was performed once during each mid- and late-lactation period, which may limit the generalizability of the findings. The focus was on tracking bacterial counts from farm bulk tanks to the final SMP product, emphasizing the importance of cow management, hygiene practices, and processing parameters in controlling bacterial levels to ensure high-quality dairy products. To narrowly target the scope of the current review, the specific mention of biofilms has excluded all studies related to raw milk and dairy product microbiota, which have been covered in other recent reviews. Although only English language publications were filtered, few publications in other languages were excluded, perhaps due to the nature of the journals indexed in the databases that were accessed. The results of this scoping review have shown that monitoring biofilms on farms and in processing facilities is an emerging topic which, despite comprehensive reviews to date, represent a small proportion of the existing literature on dairy biofilms. Biofilm formation has largely been inferred in studies that focus on examining the microbial load of raw milk and dairy products, whereas the collection of isolates can lead to screening for biofilm formation in the laboratory. Both strategies leave gaps, although they are complementary. For example, depending on the isolation process and methodology for biofilm assays (polystyrene, stainless steel, rubber, glass, medium, stain, dynamic or static biofilm model type), the proportion of biofilm-positive isolates can be highly variable (reviewed in Flint et al., 2020). Given the limitations imposed by the large number of variables affecting the entry and propagation of microorganisms in the dairy production and processing environment, it is clear that a systems approach could be applied, such as that used in microbial risk assessment for food safety. In this view, both real-world data and laboratory-driven data could be integrated into mathematical and predictive models, such as the Monte Carlo simulation models generated for extending the shelf life of fluid milk in terms of psychrotolerant spore-formers and postpasteurization contamination by gram-negative bacteria (Enayaty-Ahangar et al., 2021). Processing facilities can use this model in estimating the cost to reach a specific shelf life or to determine the shelf life that is attainable with a specific budget, according to the authors (Enayaty-Ahangar et al., 2021). Although not spe-

cific to biofilms, assessment models are being developed to evaluate milk quality according to the end product, showing the relative contribution of factors such as *Pseudomonas* level (psychrotrophs) and milk composition in comparison to season and type of farm management practices (grazing vs. indoor housing; Rey-Cadilhac et al., 2021, 2023).

Other technologies such as UV detection units for biofilm could facilitate mapping biofilms in facilities after cleaning to target sampling to areas where biofilm builds up (Aysert-Yıldız et al., 2024; Fischer et al., 2012). This type of portable equipment could avoid the effect of arbitrary surface sampling, which introduces a source of spatial variation in microbial load, reducing the probability of accurately representing the microbial composition of contamination sites.

CONCLUSIONS

Relatively few studies have focused on tracking, profiling, or monitoring biofilms across the dairy supply chain. Due to the very large number of variable factors, each study chose a focal point to provide a selective view of the overall system. This review has identified knowledge gaps of in situ biofilm research related to experimental design, methods, data analysis and control of the variables in short-term versus long-term studies. Recommendations for practices for managing biofilms on farm or in processing facilities would require a systematic review, but this might not be worthwhile until more research is available on the factors shaping microbial communities in biofilms in industrial and commercial settings. Future primary research should aim to understand the structure of biofilms to identify the on-farm practices affecting the cycle of biofilm development in milking systems.

NOTES

This research was supported by a contribution from the Dairy Research Cluster 3 (Novalait [Québec, Canada], Dairy Farmers of Ontario [Mississauga, ON, Canada], and Agriculture and Agri-Food Canada [Ottawa, Canada]) under the Canadian Agricultural Partnership AgriScience Program administered by the Dairy Farmers of Canada (Ottawa, ON, Canada), and partly by the Natural Sciences and Engineering Research Council of Canada Dairy Alliance (Ottawa, ON, Canada; grant number 566176-2021) held by G. LaPointe. Supplemental material for this article is available at https://doi.org/10.17632/gsjw4mn8gk.1. Supplemental Material S1 contains the methods and results for the scoping review literature search, and Supplemental Material S2 contains information about the 358 records in Microsoft Excel format. No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: AMS = automatic milking system; ASV = amplicon sequence variant; BTM = bulk tank milk; C&D = cleaning and disinfection; CIP = clean-in-place; DGGE = denaturing gradient gel electrophoresis; DMC = draining-matting conveyor; DMSO = dimethyl sulfoxide; ESL = extended shelf life; FSMS = food safety management system; HTS = high-throughput sequencing; ITS = internal transcribed spacer; LM = *Listeria monocytogenes*; LPC = laboratory pasteurization count; OTU = operational taxonomic unit; PTFE = polytetrafluoroethylene; QMRA = quantitative microbial risk assessment; qPCR = quantitative PCR; QS = quorum sensing; SMP = skim milk powder; SV = sequence variant; TBC = total bacterial count; VOC = volatile organic compounds; WGS = whole genome sequencing.

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ORCIDS

Gisèle LaPointe, https://orcid.org/0000-0002-4051-0983
Tara Wilson, https://orcid.org/0009-0000-6230-1368
Armin Tarrah, https://orcid.org/0000-0002-5444-7145
Mérilie Gagnon, https://orcid.org/0000-0002-0631-4946
Denis Roy https://orcid.org/0000-0002-4229-9245