Brazilian Journal of Microbiology (2020) 51:1259–1267
https://doi.org/10.1007/s42770-020-00260-x

FOOD MICROBIOLOGY - RESEARCH PAPER

Metagenomic characterization of bacterial biofilm in four food processing plants in Colombia

Arley Caraballo Guzmán [1] · Maria Isabel González Hurtado [2] · Yesid Cuesta-Astroz [1] · Giovanny Torres [1]

Received: 20 December 2019 / Accepted: 9 March 2020 / Published online: 27 March 2020

Abstract

Bacteria inside biofilms are more persistent and resistant to stress conditions found in the production environment of food processing plants, thus representing a constant risk for product safety and quality. Therefore, the aim of this study was to characterize, using 16S rRNA sequencing, the bacterial communities from biofilms found in four food processing plants (P1, P2, P3, and P4). In total, 50 samples from these four processing plants were taken after cleaning and disinfection processes. Four phyla: Proteobacteria, Firmicutes, Actinobacteria, and Bacteroides represented over 94% of the operational taxonomic units found across these four plants. A total of 102 families and 189 genera were identified. Two genera, *Pseudomonas* spp. and *Acinetobacter* spp., were the most frequently found (93.47%) across the four plants. In P1, *Pseudomonas* spp. and *Lactobacillus* spp. were the dominant genera, whereas *Lactobacillus* spp. and *Streptococcus* spp. were identified in P2. On the other hand, biofilms found in P3 and P4 mainly consisted of *Pseudomonas* spp. and *Acinetobacter* spp. Our results indicate that different bacterial genera of interest to the food industry due to their ability to form biofilm and affect food quality can coexist inside biofilms, and as such, persist in production environments, representing a constant risk for manufactured foods. In addition, the core microbiota identified across processing plants evaluated was probably influenced by type of food produced and cleaning and disinfection processes performed in each one of these.

Keywords Biofilm · Food · Next generation sequencing · Metagenomics · Microbiota

Responsible Editor: Luis Augusto Nero.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s42770-020-00260-x) contains supplementary material, which is available to authorized users.

✉ Giovanny Torres
gtorres@ces.edu.co

Arley Caraballo Guzmán
acaraballo@ces.edu.co

Maria Isabel González Hurtado
mariaisabelgonzalezh1125@gmail.com

Yesid Cuesta-Astroz
yesid.cuesta@gmail.com

[1] Colombian Institute of Tropical Medicine, CES University, Carrera 43A # 52 Sur 99, Sabaneta, Colombia

[2] Department of Research and Development, Zenú Food Industry, Carrera 64C # 104-3, Medellín, Colombia

Introduction

Food product quality and safety depends on the microbial load and absence of pathogenic bacteria found in production areas [1]. Therefore, cleaning and disinfection processes (C&D) play a key role in the quality control system of food processing plants [2]. Surfaces in the food processing areas that come in contact with products may act as a contamination source of spoilage and pathogenic bacteria [3]. Consequently, these bacteria may reach products during food production directly from conveyor belts, slicers, utensils, or biofilms on surfaces [4, 5].

Biofilms can be an important source of food contamination and lead to economic losses due to spoilage or issues with food safety [6, 7]. Bacteria forming biofilms are protected against environmental stress conditions, such as cleaning, disinfection, drying, high temperature, and low-nutrient conditions, which ensure bacterial viability and persistence in the food production areas [8, 9]. The ability of some food-associated pathogens to

attach to food processing surfaces and to form biofilm has been previously reported for pathogens such as *Listeria monocytogenes* [10] and *Staphylococcus aureus* [11], as well as for spoilage bacteria such as *Pseudomonas* spp. and *Lactobacillus* spp. [12, 13].

Several studies addressing biofilms in the food industry have been focused on specific pathogenic bacteria and have contributed important knowledge about their biology and prevalence [14, 18, 20]. However, the impact of bacterial interactions in food processing environments must be taken into account, since these interactions may promote and improve biofilm formation [15, 16]. Interactions in multispecies biofilms enhance protection of species that are less tolerant to stress factors and have low ability of biofilm formation [17]. To date, the greater ability of some bacterial genera to produce biofilms, compared with others, has been well documented. For instance, some strains of *Pseudomonas* spp. have been shown to enhance biofilm formation by *L. monocytogenes* [18]. Likewise, Oliver et al. (2010) indicated that *Escherichia coli* O157:H7 strain was protected by a biofilm formed by an *Acinetobacter calcoaceticus* strain isolated from meat processing facilities [19]. Furthermore, both *Pseudomonas* and *Acinetobacter* genera are bacteria that can be frequently found on food processing surfaces [9, 20].

Traditionally, the study of microorganisms has been concentrated on analyzing single species in pure culture [12]. However, a significant number of microorganisms cannot be isolated and studied in the laboratory with current conventional microbiological methods. Microbiological culture generally favors the recovery of organisms adapted to laboratory conditions; however, these may not necessarily to be the dominant or the most influential organisms in the environment [14]. Therefore, in order to better understand microbial communities, there is a need for traditional techniques (microbiological culture) to be supplemented with other culture-independent strategies such as metagenomics, which allows the identification of microorganisms recovered directly from their own environment. Using different DNA markers such as 16S, 18S, and ITS (internal transcribed spacer), the metagenomic approach allows the simultaneous study of many organisms without the need of individually isolating each one of them [21].

Identifying bacteria that form biofilms is important not only to determine the resident "*Bacteriota*" and to understand microbial communities but also to evaluate sanitation processes and microbiological sampling in food processing industries [22, 23]. Therefore, the aim of this study was to characterize, using 16S rRNA sequencing, the bacterial communities from biofilms found in four food processing plants.

Materials and methods

Food processing plants

Four different food processing plants located in two of the departments of Colombia were evaluated. The plants were identified as P1, P2, P3, and P4. P1 and P2 processed ready-to-eat meal products such as lasagna, ravioli, spaghettis, and pizzas. On the other hand, P3 and P4 processed meat products such as mortadella, ham, meatballs, and sausage. The average temperature and humidity of the plants were 12.5 °C and 67%, respectively. A quaternary ammonium-based product was used for the disinfection process in all plants.

Sampling

One hour after C&D processes, a total of 50 samples were collected from packing and production areas. The samples on each plant were taken the same day, but on different days among plants. The sampled areas were in contact with the food produced in the four plants described above. Sampled surface materials were stainless steel 39 (78%), rubber 6 (12%), aluminum 2 (4%), sailcloth 2 (4%), and iron 1 (2%). Biofilms were tested according to two criteria; the first criterion was the observation of changes in the appearance of surfaces from mesons or machines. Those changes were the presence of pale yellow or colorless aggregates (flocks or granules) and plates (opaque) [24]. The second criterion was the confirmation of visually identified biofilms using BioFinder reagent (itram® Hygiene, Spain), following manufacturer's instructions. Sampling was carried out using sterile FloQ Swabs (COPAN, Italy). Every sample was taken by scraping and brought to 1 ml of sterile water nuclease-free (Roche, Germany), which were transported at 4 °C at the laboratory and stored at − 20 °C until processing (Table 1).

DNA extraction

The collected bacteria were harvested by centrifugation at 14,000×*g* for 10 min at 4 °C. DNA extraction was performed

Table 1 Number of points sampled by plant

Plant	Number of points sampled by area		Total samples
	Packing	Production	
P1	0	16	16
P2	0	14	14
P3	8	0	8
P4	3	9	12

0: No biofilm detected across this area

using High Pure Template kit (Roche, Germany), following manufacturer's instructions. Finally, samples were frozen at − 80 °C until analysis.

Amplicon-based metagenomic profiling of bacterial communities

Bacterial identification was performed using next-generation sequencing (NGS) using the Illumina Miseq platform and targeting V4 hypervariable region of the 16S rRNA gene. DNA sequencing was outsourced to Research Testing Laboratory (RTL. Lubbock, TX, USA). The following primers were used: 515F-806R as described in a previous study [25]. Three steps were followed after result analysis; denoising to correct error reads using USEARCH [26]. Then, the reads were organized according to size in order to eliminate duplicated sequences. Chimeric sequences were eliminated using the UCHIME software [27]. Operational taxonomic unit (OTU) selection was performed using the UPARSE OTU selection algorithm (identity threshold 97%) [28] to classify the large number of clusters into OTUs. In order to determine the taxonomic information, Greengenes database was used.

Analysis of the bacterial composition

Microbial community composition comparisons were based on pairwise dissimilarity measurements. A variance-stabilizing transformation for relative abundance matrix was applied for dispersion reduction; values were showed in $\arcsin\sqrt{x}$ [29].

To assess difference in bacterial community composition, richness and abundance were studied according to alpha diversity by using Shannon index and β-diversity based on SIMPER analysis in order to know the bacteria that contributed more to microbiota composition between plants. We also investigated indicator bacteria within plants through IndicSpecies. Hierarchical clustering was performed on the transformed dataset based on the Bray-Curtis (B-C). These analyses were performed using the R software version 3.5.1.

Venn diagram for description OTUs at family and gender level in each of the four plants was performed using Venny 2.1 program (http://bioinfogp.cnb.csic.es/tools/venny/).

Results

Taxonomic profiling of bacterial communities

A total of 1,426,157 16S rRNA sequences were generated by NGS from the samples collected at the four processing plants studied. Of these, 87.7% (1,250,157) were clustered into some OTUs. The remaining 12.3% (175,585) had no hits associated with OTUs. A total of four samples did not yield amplified product (two samples in P1 and two samples in P3), probably due to DNA degradation, presence of an inhibitor substance in the sample, or that there was no bacterial DNA.

Four phyla were dominant and constituted more than 94% of the identified phyla across all four processing plants: Proteobacteria, Firmicutes, Actinobacteria, and Bacteroides. The most frequent phylum at P1, P3, and P4 was Proteobacteria, representing 47.3%, 51.7%, and 45.3% of OTUs, respectively. On the other hand, in P2, the dominating phylum was Firmicutes with 50.3% of OTUs (Table 2).

Identification of exclusive and core microbiota across food processing plants

Our analysis identified a total 102 families and 189 genera. Of the 102 families identified, 37 (36.3%) were common across all plants, and 22 (21.6%) were specific. P3 had the highest number of unique families, nine (8.8%), followed by P1, P2, and P4 with six (6.9%), four (3.9%), and two (2%), respectively (Fig. 1a). The most frequent families found were *Pseudomonaceae* and *Moraxellaceae* with a 93.5% (43/46) in overall plants, followed by *Lactobacillaceae* with 89.1% (41/46), and *Streptococcaceae* family with 78.2% (36/46) (Supplementary S1).

Of the 189 OTUs identified at the genus level, 50 (24.5%) genera were shared across all four plants, whereas 70 (37.0%) were specific, being P1 the one with the most unique genera, in total 26 (13.8%), followed by P3 with 24 (12.7%), and P2 and P4 exhibited the same number, 10 (5.3%) (Fig. 1b).

Dominant genera identified in biofilm

Regarding the relative abundance and frequency analysis, *Pseudomonas* spp. had the highest frequency and relative abundance. This genus was identified in 35.7% of samples from P1, and 50% for P3, and had an average relative abundance of 50.5% and 43.9%, at P1 and P3, respectively. In addition, SIMPER analysis showed that *Pseudomonas* genus was more common between P1–P2 and P1–P3, with highest similarity percentage, while in P2, *Lactobacillus* genus was the dominating bacteria with a frequency of 57.1%, and an average relative abundance of 38.6%. Likewise, this genus was the one contributing the most to the similarity between P2–P3. In P4, the highest percentage was for *Acinetobacter* spp., 66.7%, which had an average relative abundance of 72.9%, contributing to most similarity between P2–P4 and P3–P4 (Fig. 2) (Supplementary S2).

***Resident lactic acid bacteria across food processing plants*:** Lactic acid bacteria (LAB) were present in all of the analyzed

Table 2 Main OTUs at phylum level found across the four plants

Plant	Actinobacteria (%)	Proteobacteria (%)	Firmicutes (%)	Bacteroides (%)	Others (%)
P1	10.5	*47.3*	32.5	6.1	3.6
P2	5.1	33.8	*50.3*	4.8	6.0
P3	16.5	*51.7*	19.6	8.3	3.9
P4	16.6	*45.3*	22.8	9.6	5.7

Number in italics indicates the phylum most frequency in each plant

food processing plants. For example, *Streptococcaceae* family was the most prevalent in P1 and was represented by

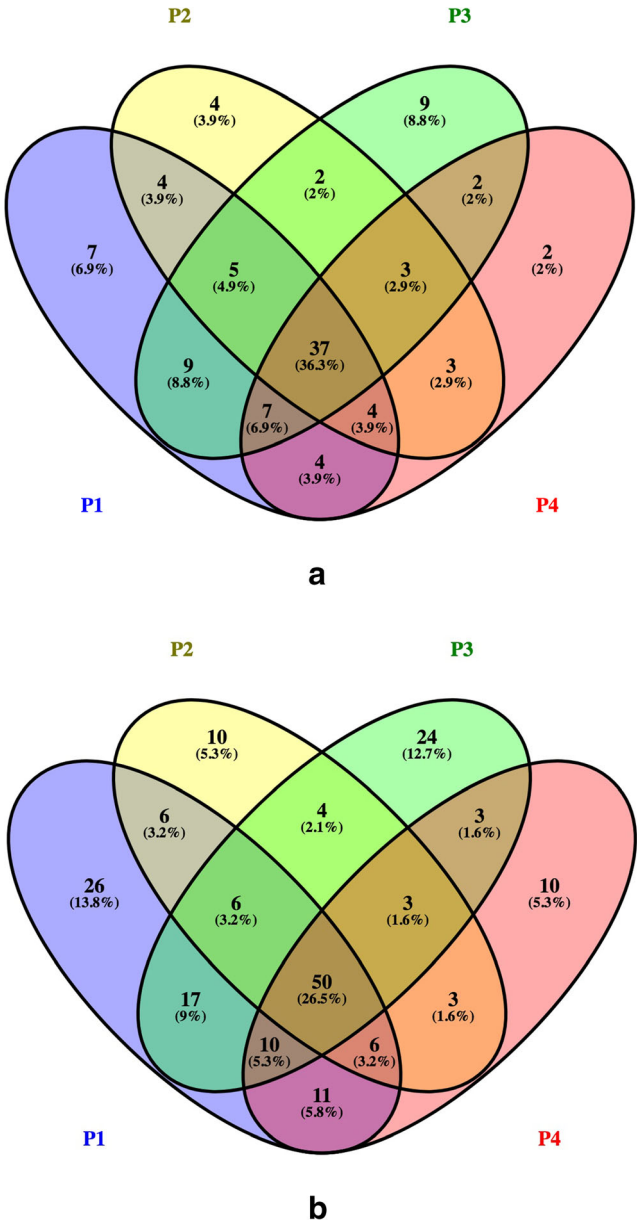

Fig. 1 Venn diagrams. The letter and number together outside the ovals and their colors represent each plant. The numbers in intersections indicate common families or genera among plants. The numbers outside of the intersections are the families or genera found only in those plants. **a** Families identified across plants. **b** Genera identified across plants

Lactococcus spp. and *Streptococcus* spp., both with the same frequency (78.6%). *Lactobacillaceae* was the second family most frequently found at P1, being *Lactobacillus* spp. (71.4%) and *Leuconostoc* spp. (64.3%) the genera most frequently identified; however, *Leuconostoc* was recognized as the indicator species at P1 ($P < 0.05$) (Supplementary S3).

Likewise, two families were dominant in P2, *Lactobacillaceae* and *Streptococcaceae*. In this plant, *Lactobacillus* and *Streptococcus* genera were the most frequently identified (78.6%). However, *Lactobacillus* was the indicator species for P2 ($P < 0.05$) (Supplementary S3). On the other hand, the *Streptococcaceae* family with the *Streptococcus* genus (83.3%) and the *Alicyclobacillaceae* family with *Alicyclobacillus* genus (66.6%) were the predominating ones in P3. Unlike the other plants, P4 was where less LAB were identified, being *Alicyclobacillus* genus the most frequent (33.3%).

Enterobacteriaceae across plant: *Enterobacteriaceae* family members were also identified. *Escherichia* spp., *Enterobacter* spp., *Cronobacter* spp., *Hafnia* spp., *Morganella* spp., and *Serratia* spp. were some of the genera found in this study. *Escherichia* spp. were present in all plants, being the most frequently found in P1 (64.2%), followed by P2 (57.1%), P3 (50%), and P4 (25%).

According to our results, pathogenic bacteria associated with foodborne diseases, such as *L. moncytogenes*, *Salmonella* spp., *S. aureus*, *Campylobacter* spp., or *Bacillus subtilis*, were not identified in our samples.

Diversity analysis

Analysis of alpha diversity at the family level according to the Shannon index showed that the most diverse plant was P3 with an average of 1.82, followed by P1 with 1.59, P2 with 1.46, and P4 with 1.20 (Supplementary S4). Moreover, P3 was the plant with the greatest number of indicator species, among these, *Sphingomona* spp., which exhibited the highest strength (90.8%) and statistical significance ($P < 0.05$).

Beta diversity under Bray-Curtis analysis showed apparently two large groups. P1 and P2 were clustered within group one, whereas the P3 and P4 were clustered within group two (Fig. 3).

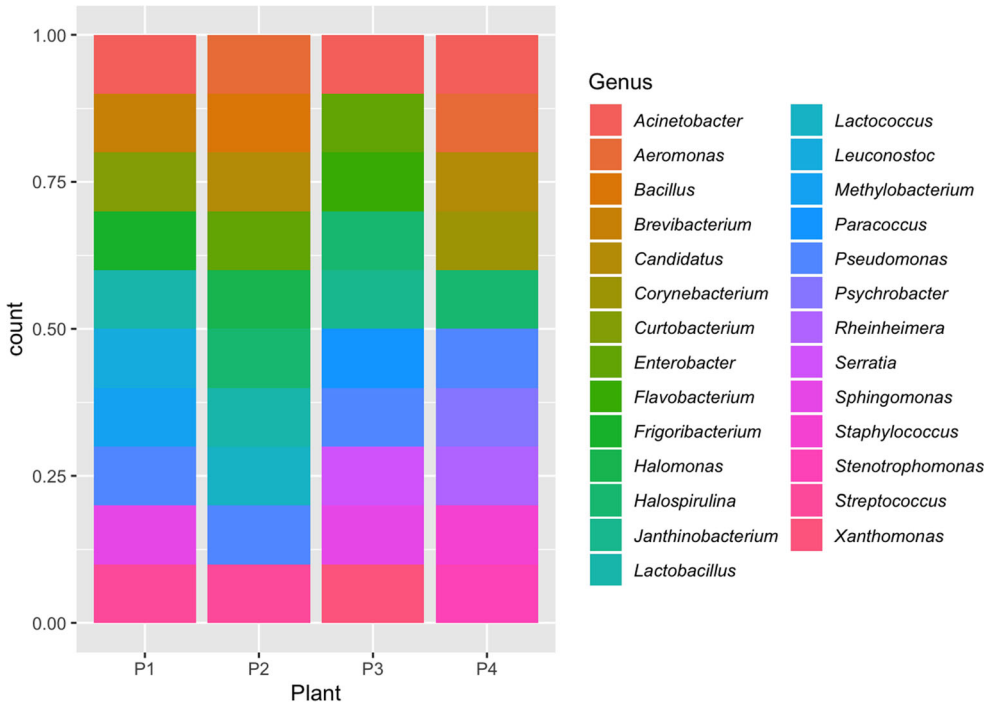

Fig. 2 Ten main genera found in each plant according to relative abundance. P1, P2, P3, and P4 represent each food processing plant (X–axis). Color boxes represent bacterial abundance (Y–axis)

Discussion

The implementation of next-generation sequencing methodologies has improved the study of microbial diversity on different environments [30]. These tools have complemented conventional microbiological techniques [31], which are insufficient to study the biodiversity of microorganisms, since most bacteria cannot be isolated and identified by microbiological culture [32]. In addition, evidence has shown that the absence or inappropriate use of neutralizing substances, as well as unsuitable growing conditions (incubation temperature and culture media), could lead to an underestimation of the

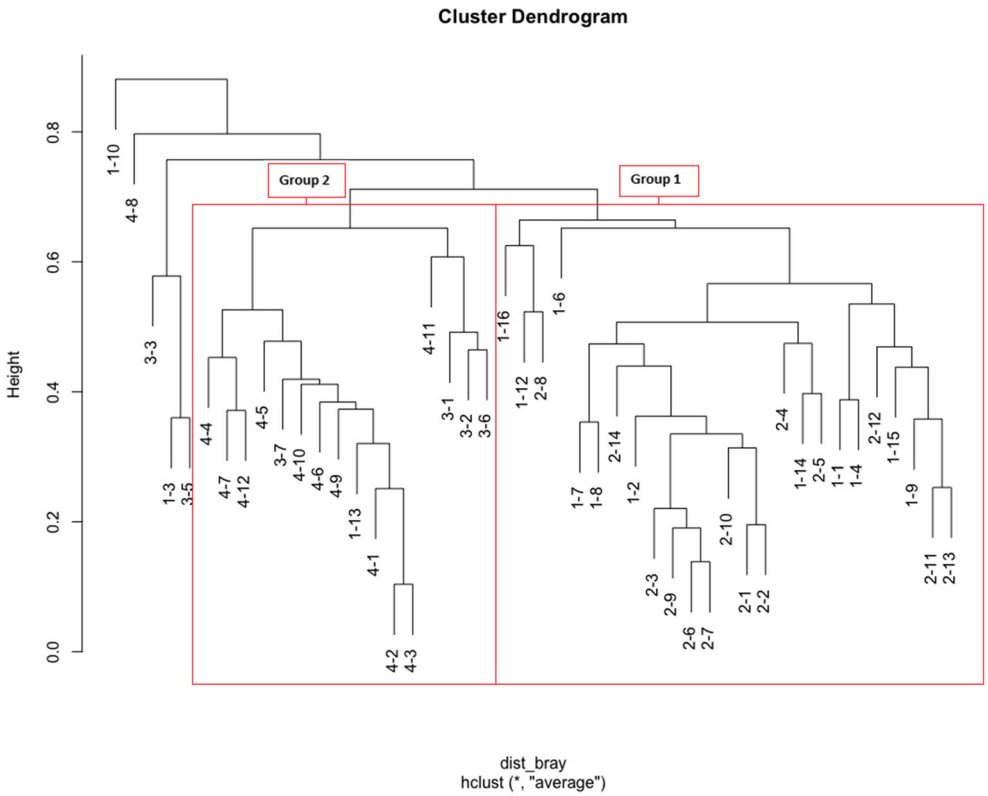

Fig. 3 Communities at families level by Bray-Curtis analysis. Red square indicates the families clustered into two groups. The first number corresponds to plant (1-X); the second number indicates the point code sampled (X-1)

bacterial diversity and abundance [33]. The metagenomic approach implemented in this study allowed to determine both diversity and abundance of bacteria that were part of the analyzed biofilms [34, 35].

It has been previously described that resident microbiota in food plants will depend on environmental conditions (air flow, temperature, and humidity), C&D process, food origin (animal, vegetable), geographical region, and even on human microbiota [36]. Beta diversity analysis at the family level showed that bacterial communities forming biofilms found in these processing plants could be clustered into two large groups. This clustering could be explained by the foods processed in each plant, since in P1 and P2 (group 2), ready-to-eat meal products (e.g., lasagnas and pizzas) were manufactured, while in P3 and P4 (group 1), both processed meat products (e.g., sausages, ham, and mortadella). Furthermore, processing plants included in the same group had raw materials in common (e.g., dairy products in P1 and P2; meat in P3 and P4), suggesting the possible influence of these materials on resident microbiota.

On the other hand, *Pseudomonas* spp. were a common bacteria identified across plants. This bacterium is important in the industrial environments due to the negative impact it may have on food quality, its ability to form biofilm, and to survive and grow under harmful conditions [2]. Some strains of *Pseudomonas* spp. have the ability to grow at low temperatures (4–12 °C) [37] and to tolerate some disinfectants, especially quaternary ammonium compounds [18], which are broadly used during the sanitation process in the food industry [38], and all plants involved in this study used this product during the disinfection process. Moreover, some studies have demonstrated that the association of *Pseudomonas* spp. with other bacteria, such as *L. monocytogenes* [18] and *S. aureus* [39], favors the development of more compact biofilm, which probably makes them more resistant to C&D process [40]. However, we did not find these bacteria cohabiting in the studied biofilms. On the other hand, we found cohabitation with lactic acid bacteria, which is an association that has been previously identified in biofilms [41].

In this study, *Pseudomonas* spp. were the dominant bacteria in biofilms in samples from P1 to P3 (more frequency and abundance), in comparison with other bacteria that cohabited these plants. Our results are in agreement with previous studies in which this bacterium was found to be the most predominant one in samples from food contact surfaces [42]. Some authors have reported the dominance of *Pseudomonas* spp. over other bacteria during biofilm formation in vitro. Langsrud et al., (2016) showed that *Pseudomonas* spp. always prevailed on the formed biofilms when was used a cocktail of different bacterial species, between 11 and 14 strains, isolated from food processing plants [9].

Acinetobacter spp. were another bacterium that predominated in the biofilms studied. It was present in most of the points sampled across the four food processing plants. This genus is often reported as a persistent bacterium after C&D process, not only to produce biofilm for its ability but also to survive under industrial environmental conditions [43]. *Acinetobacter* spp. may form biofilm under dynamic conditions, which gives a growth advantage over other bacteria. A study showed that *E. coli* O157:H7 strain, a bacterium without the ability to form biofilm under dynamic conditions, increased its count up to 400 times after it was embedded in the biofilm formed by *A. calcoaceticus* [19]. Our results showed that *Acinetobacter* spp. were the dominant genus in biofilms from P4, both in frequency and abundance. Nonetheless, according to previous studies, this behavior could be transient, since during an in vitro experiment, the authors observed that the predominance changed as the biofilm matured, initially identifying the predominance of *Acinetobacter* spp. (first 4 days) and of *Pseudomonas putida* at the end of the study [22]. The competition for limiting nutrient sources could be the cause of transitional microbial compositions of biofilms. Previous studies have demonstrated that both *Acinetobacter* spp. and *Pseudomonas* spp. can often be found in the same environment because these bacteria have similar characteristics, such as biofilm forming ability and antimicrobial resistance [44]. However, *Acinetobacter* spp. are less frequent in the production environment and have less impact on product quality [33].

Unlike P1, P3, and P4, where Gram-negative bacteria were the predominant ones, in P2, the Gram-positive bacterium, *Lactobacillus* spp., was the most frequent, being the only LAB with this abundance. This fact was enough to generate a change at the phylum level, since P2 was the only plant where the Firmicutes presented a very high proportion. LAB is a group of microorganisms capable of growing at low temperatures, and some species are relatively tolerant to stress conditions [45], thus providing them with a high food spoilage ability [46, 47]. Sample points where *Lactobacillus* genus predominated neither *Pseudomonas* spp. nor *Acinetobacter* spp. were present, at least among the top five more abundant bacteria. Therefore, Gram-positive bacteria prevailed in most sample points from P2 (72%), suggesting dominance and persistence of these bacteria over Gram-negative bacteria. Evidence has shown that LAB are predominant in dairy industries, where they are used as a starter culture [33]. This fact could explain the results obtained in the P2 because in that plant, dairy products (cheese and milk) were used as raw material. However, this was not observed in P1 samples, despite also having used the same dairy products.

Enterobacteriaceae is a bacterial family of great importance in the food industry because they are indicators of inappropriate hygiene practices. Bacteria such as *Salmonella* spp. and *E. coli* O157:H7 are considered foodborne pathogens, and some species are recognized as food spoilers. [48–50]. The main genera of the

Enterobacteriaceae family found in this study were *Escherichia* spp. and *Serratia* spp. In agreement with our results, some authors have also reported on *Escherichia* genus in food processing environments [51]. High prevalence of *Serratia* spp. agrees with that reported in other studies, where this genus was more identified in different food processing plants [37]. Interestingly, a study in a milk processing plant showed that *Serratia* spp. presented greater ability of adherence and biofilm formation than *Pseudomonas* spp. [52, 53]. In addition, it has been evidenced that this bacterium may present high resistance to disinfectants and ability to grow at low temperatures, which allows it to compete with other psychrotrophic bacteria [53].

The resident microbiota in a specific environment has been also related with type of food, its composition, and nutrient availability for bacteria [54]. For instance, a study carried out in a dairy plant environment reported a core microbiota shaped by LAB, where *Lactobacillus* spp. showed a negative correlation with *Pseudomonas* spp. and *Acinetobacter* spp. According to the authors, these results could indicate that persistence of LAB in the environment helps to control the food spoilers [55]. In this study, similar results were obtained from P1, since LABs, specifically the *Lactobacillus* genus, were the predominant bacteria (57.1%) in most biofilms evaluated. In contrast to dairy plant environments, a study reported that bacteria, such as *Psychrobacter*, *Rhodococcus*, *Leuconostoc*, and *Yersinia* genera, had been detected in meat processing plant surfaces, with *Yersinia* spp. being the most abundant [45]. Unlike these results, we found *Pseudomonas* and *Acinetobacter* as the predominant genera across meat processing plants, P3 and P4. This is in agreement with other studies of bacterial spoilers in meat plant environments, where these bacteria predominated on the evaluated surface [12, 56].

Conclusions

The biofilms characterized from samples from the four processing plants showed *Pseudomonas* spp. and *Acinetobacter* spp. as the dominant bacteria, which are broadly recognized as strong biofilm formers. However, these bacteria cohabited with spoiler microorganisms of interest to the food industry such as *Leuconostoc* spp., *Lactobacillus* spp., *Streptococcus* spp., and Enterobacteriaceae.

Although in our study, foodborne pathogens were not detected in the biofilms, we cannot ignore the protector and synergic effect that offer the biofilms for these microorganisms, which represents a constant risk for the safety and quality of processed foods.

The mapping of the bacteria that inhabit on surface of the food producing areas is necessary to estimate the microbiological risk of each environment and to optimize the C&D protocols.

Funding information This study was supported by COLCIENCIAS grant 55965, the Research and Development, Zenu Food Industry Medellín, and the Colombia Research group Colombian Institute of Tropical Medicine and CES University.

References

1. Norman MM, Schilling W, Gravani RB (2018) Principles of food sanitation [Internet], 6th edn. Springer, Berlin 422 p. Disponível em: https://www.springer.com/la/book/9783319671642#aboutBook
2. Esbelin J, Santos T, Hébraud M (2018) Desiccation: an environmental and food industry stress that bacteria commonly face. Food Microbiol 69:82–88
3. Ostrov I, Sela N, Belausov E, Steinberg D, Shemesh M (2019) Adaptation of Bacillus species to dairy associated environment facilitates their biofilm forming ability. Food Microbiol 82:316–324
4. Ferreira V, Wiedmann M, Teixeira P, Stasiewicz MJ (2014) *Listeria monocytogenes* persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. J Food Prot 77(1):150–170
5. Møretrø T, Langsrud S, Heir E (2013) Bacteria on meat abattoir process surfaces after sanitation: characterisation of survival properties of *Listeria monocytogenes* and the commensal bacterial flora. Adv Microbiol 03(03):255–264
6. Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F (2011) Resistance of bacterial biofilms to disinfectants: a review. Biofouling 27(9):1017–1032
7. Saucier L (2016) Microbial spoilage, quality and safety within the context of meat sustainability. Meat Sci 120:78–84
8. de Souza EL, Meira QGS, de Medeiros BI, Athayde AJAA, da Conceição ML, de Siqueira Júnior JP (2014) Biofilm formation by *Staphylococcus aureus* from food contact surfaces in a meat-based broth and sensitivity to sanitizers. Braz J Microbiol 45(1):67–75
9. Langsrud S, Moen B, Møretrø T, Løype M, Heir E (2016) Microbial dynamics in mixed culture biofilms of bacteria surviving sanitation of conveyor belts in salmon-processing plants. J Appl Microbiol 120(2):366–378
10. Schirmer BCT, Heir E, Møretrø T, Skaar I, Langsrud S (2013) Microbial background flora in small-scale cheese production facilities does not inhibit growth and surface attachment of *Listeria monocytogenes*. J Dairy Sci 96(10):6161–6171
11. Gutiérrez D, Delgado S, Vázquez-Sánchez D, Martínez B, Cabo ML, Rodríguez A et al (2012) Incidence of *Staphylococcus aureus* and analysis of associated bacterial communities on food industry surfaces. Appl Environ Microbiol 78(24):8547–8554
12. Brightwell G, Boerema J, Mills J, Mowat E, Pulford D (2006) Identifying the bacterial community on the surface of Intralox belting in a meat boning room by culture-dependent and culture-independent 16S rDNA sequence analysis. Int J Food Microbiol 109(1–2):47–53
13. Giaouris E, Heir E, Hébraud M, Chorianopoulos N, Langsrud S, Møretrø T et al (2014) Attachment and biofilm formation by foodborne bacteria in meat processing environments: causes, implications, role of bacterial interactions and control by alternative novel methods. Meat Sci 97(3):298–309

14. Winkelströter LK, Teixeira FB d R, Silva EP, Alves VF, De Martinis ECP (2014) Unraveling microbial biofilms of importance for food microbiology. Microb Ecol 68(1):35–46
15. Liu NT, Nou X, Bauchan GR, Murphy C, Lefcourt AM, Shelton DR et al (2015) Effects of environmental parameters on the dual-species biofilms formed by *Escherichia coli* O157:H7 and *Ralstonia insidiosa*, a strong biofilm producer isolated from a fresh-cut produce processing plant. J Food Prot 78(1):121–127
16. Tarifa MC, Lozano JE, Brugnoni LI (2015) Dual-species relations between *Candida tropicalis* isolated from apple juice ultrafiltration membranes, with *Escherichia coli* O157:H7 and *Salmonella* sp. J Appl Microbiol 118(2):431–442
17. Burmølle M, Ren D, Bjarnsholt T, Sørensen SJ (2014) Interactions in multispecies biofilms: do they actually matter? Trends Microbiol 22(2):84–91
18. Giaouris E, Chorianopoulos N, Doulgeraki A, Nychas G-J (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
19. Olivier H, Heir E, Langsrud S, Åsli AW, Møretrø T (2010) Enhanced surface colonization by *Escherichia coli* O157:H7 in biofilms formed by an *Acinetobacter calcoaceticus* isolate from meat-processing environments. Appl Environ Microbiol 76(13):4
20. Mertz AW, Koo OK, O'Bryan CA, Morawicki R, Sirsat SA, Neal JA et al (2014) Microbial ecology of meat slicers as determined by denaturing gradient gel electrophoresis. Food Control 42:242–247
21. Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng J-F et al (2013) Insights into the phylogeny and coding potential of microbial dark matter. Nature 499(7459):431–437
22. Fagerlund A, Møretrø T, Heir E, Briandet R, Langsrud S (2017) Cleaning and disinfection of biofilms composed of *Listeria monocytogenes* and background microbiota from meat processing surfaces. Appl Environ Microbiol
23. Verran J, Redfern J, Smith LA, Whitehead KA (2010) A critical evaluation of sampling methods used for assessing microorganisms on surfaces. Food Bioprod Process 88(4):335–340
24. Chmielewski RAN, Frank JF (2003) Biofilm formation and control in food processing facilities. Compr Rev Food Sci Food Saf 2(1): 22–32
25. Yang B, Wang Y, Qian P-Y (2016) Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. BMC Bioinformatics [Internet]. [citado 17 de fevereiro de 2018];17(1). Disponível em: http://www.biomedcentral.com/1471-2105/17/135
26. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinforma Oxf Engl 26(19):2460–2461
27. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16):2194–2200
28. Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10(10):996–998
29. Ramette A (2007) Multivariate analyses in microbial ecology. FEMS Microbiol Ecol 62(2):142–160
30. Culligan EP, Sleator RD, Marchesi JR, Hill C (2014) Metagenomics and novel gene discovery: promise and potential for novel therapeutics. Virulence 5(3):399–412
31. Ismaïl R, Aviat F, Michel V, Le Bayon I, Gay-Perret P, Kutnik M et al (2013) Methods for recovering microorganisms from solid surfaces used in the food industry: a review of the literature. Int J Environ Res Public Health 10(11):6169–6183
32. Janda JM, Abbott SA (2014) Culture-independent diagnostic testing: have we opened Pandora's box for good? Diagn Microbiol Infect Dis 80(3):171–176
33. Møretrø T, Langsrud S (2017) Residential bacteria on surfaces in the food industry and their implications for food safety and quality: residential bacteria in food industry. Compr Rev Food Sci Food Saf 16(5):1022–1041
34. Flores GE, Bates ST, Caporaso JG, Lauber CL, Leff JW, Knight R et al (2013) Diversity, distribution and sources of bacteria in residential kitchens: bacterial diversity of residential kitchens. Environ Microbiol 15(2):588–596
35. Jagadeesan B, Gerner-Smidt P, Allard MW, Leuillet S, Winkler A, Xiao Y et al (2019) The use of next generation sequencing for improving food safety: translation into practice. Food Microbiol 79:96–115
36. Kembel SW, Meadow JF, O'Connor TK, Mhuireach G, Northcutt D, Kline J et al (2014) Architectural design drives the biogeography of indoor bacterial communities. White BA, organizador. PLoS One 9(1):e87093
37. Zhang Y, Wei J, Yuan Y, Yue T (2019) Diversity and characterization of spoilage-associated psychrotrophs in food in cold chain. Int J Food Microbiol 290:86–95
38. Gadea R, Fernández Fuentes MÁ, Pérez Pulido R, Gálvez A, Ortega E (2017) Effects of exposure to quaternary-ammonium-based biocides on antimicrobial susceptibility and tolerance to physical stresses in bacteria from organic foods. Food Microbiol 63:58–71
39. Abdallah M, Khelissa O, Ibrahim A, Benoliel C, Heliot L, Dhulster P et al (2015) Impact of growth temperature and surface type on the resistance of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms to disinfectants. Int J Food Microbiol 214:38–47
40. Puga CH, Orgaz B, SanJose C (2016) *Listeria monocytogenes* impact on mature or old Pseudomonas fluorescens biofilms during growth at 4 and 20°C. Front Microbiol 7:134
41. Lapointe C, Deschênes L, Ells TC, Bisaillon Y, Savard T (2019) Interactions between spoilage bacteria in tri-species biofilms developed under simulated meat processing conditions. Food Microbiol 82:515–522
42. Stellato G, La Storia A, Cirillo T, Ercolini D (2015) Bacterial biogeographical patterns in a cooking center for hospital foodservice. Int J Food Microbiol 193:99–108
43. Carvalheira A, Casquete R, Silva J, Teixeira P (2017) Prevalence and antimicrobial susceptibility of Acinetobacter spp. isolated from meat. Int J Food Microbiol 243:58–63
44. Hansen SK, Haagensen JAJ, Gjermansen M, Jørgensen TM, Tolker-Nielsen T, Molin S (2007) Characterization of a *Pseudomonas putida* rough variant evolved in a mixed-species biofilm with Acinetobacter sp. strain C6. J Bacteriol 189(13):4932–4943
45. Li J, Yang X, Shi G, Chang J, Liu Z, Zeng M (2019) Cooperation of lactic acid bacteria regulated by the AI-2/LuxS system involve in the biopreservation of refrigerated shrimp. Food Res Int 120:679–687
46. Hultman J, Rahkila R, Ali J, Rousu J, Björkroth KJ (2015) Meat processing plant microbiome and contamination patterns of cold-tolerant bacteria causing food safety and spoilage risks in the manufacture of vacuum-packaged cooked sausages. Drake HL, organizador. Appl Environ Microbiol 81(20):7088–7097
47. Perpetuini G, Pham-Hoang BN, Scornec H, Tofalo R, Schirone M, Suzzi G et al (2016) In *Lactobacillus pentosus*, the olive brine adaptation genes are required for biofilm formation. Int J Food Microbiol 216:104–109
48. Gounadaki AS, Skandamis PN, Drosinos EH, Nychas G-JE (2008) Microbial ecology of food contact surfaces and products of small-scale facilities producing traditional sausages. Food Microbiol 25(2):313–323
49. Montgomery NL, Banerjee P (2015) Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in biofilms by pulsed ultraviolet light. BMC Res Notes [Internet]. [citado 28 de agosto de 2019];8(1). Disponível em: http://www.biomedcentral.com/1756-0500/8/235

50. Tadepalli S, Bridges DF, Driver R, Wu VCH (2018) Effectiveness of different antimicrobial washes combined with freezing against Escherichia coli O157:H7, *Salmonella* Typhimurium, and Listeria monocytogenes inoculated on blueberries. Food Microbiol 74:34–39
51. Holah JT, Bird J, Hall KE (2004) The microbial ecology of high-risk, chilled food factories; evidence for persistent Listeria spp. and *Escherichia coli* strains. J Appl Microbiol 97(1):68–77
52. Cleto S, Matos S, Kluskens L, Vieira MJ (2012) Characterization of contaminants from a sanitized milk processing plant. PLoS One 7(6):e40189
53. Langsrud S, Møretrø T, Sundheim G (2003) Characterization of *Serratia marcescens* surviving in disinfecting footbaths. J Appl Microbiol 95(1):186–195
54. Marchand S, De Block J, De Jonghe V, Coorevits A, Heyndrickx M, Herman L (2012) Biofilm formation in milk production and processing environments; Influence on Milk Quality and Safety. Compr Rev Food Sci Food Saf 11(2):133–147
55. Stellato G, De Filippis F, La Storia A, Ercolini D (2015) Coexistence of lactic acid bacteria and potential spoilage microbiota in a dairy processing environment. Elkins CA, organizador. Appl Environ Microbiol 81(22):7893–7904
56. De Filippis F, La Storia A, Villani F, Ercolini D (2013) Exploring the sources of bacterial spoilers in beefsteaks by culture-independent high-throughput sequencing. Virolle M-J, organizador. PLoS One 8(7):e70222