



Comparative analysis of biofilms in the meat and poultry processing industry: taxonomy and interactions

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ABSTRACT

This study aimed to compare the prevalence, taxonomic structure, and interactions of microbial communities in biofilms formed on surfaces in a meat processing plant (MPP) and a poultry processing plant (PPP). Using transmission electron microscopy and high-throughput sequencing of the 16S rRNA V3-V4 region, biofilms were detected in 25% of MPP scrape samples (2/8) and in 85.7% of PPP scrape samples (6/7). Taxonomic analysis revealed the dominance of *Proteobacteria*, *Bacteroidota*, *Actinobacteria*, and *Firmicutes* in biofilms from both facilities, with marked heterogeneity in the MPP (Chao1 index: 336.8–697.8). Network association of microorganisms has identified a synergy between the genera *Flavobacterium*, *Acinetobacter*, and *Psychrobacter*, and an antagonism between the *Marisediminicola* and *Pseudomonas/Acinetobacter*, which highlights the complexity of inter-microbial interactions in industrial settings.

1. Introduction

The microbial ecology of industrial food processing environments is a critical determinant of product safety and quality. Biofilms formed on equipment and surfaces in meat and poultry processing plants serve as reservoirs for spoilage microorganisms and potential pathogens, posing significant risks to both product shelf life and consumer health (Giaouris *et al.*, 2014). Despite advances in sanitation practices, persistent microbial contamination remains a challenge, particularly in facilities with complex workflows and varying environmental conditions. Understanding the composition, diversity, and interactions of microbial communities in these environments is essential for developing targeted disinfection strategies and mitigating contamination risks (Simões *et al.*, 2010).

Meat and poultry processing plants are characterized by unique environmental niches, including low temperatures, high humidity, and organic residues,

which favor the proliferation of psychrotrophic and biofilm-forming bacteria (Sofos, 2008). These microorganisms, such as *Pseudomonas*, *Psychrobacter*, and *Brochothrix*, are well-known agents of food spoilage, capable of degrading proteins and lipids even under refrigeration (Remenant *et al.*, 2015). Additionally, biofilms protect embedded microbes from routine sanitation, enabling recurrent contamination (Bridier *et al.*, 2015). While traditional microbiological methods have provided foundational insights, modern molecular approaches, such as 16S rRNA amplicon sequencing, offer unprecedented resolution for mapping microbial diversity and ecological dynamics in these complex systems (De Filippis *et al.*, 2017).

Previous studies have focused on specific pathogens (e.g., *Listeria monocytogenes*, *Salmonella* spp.) or spoilage organisms in food matrices (Carpentier & Cerf, 2011; Walia *et al.*, 2017), but fewer have systematically analyzed biofilm communities

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on industrial surfaces (Silva et al., 2024). Furthermore, comparative analyses between meat and poultry processing environments are scarce, despite differences in raw materials, processing stages, and sanitation protocols (Stellato et al., 2016). This study addresses these gaps by investigating the taxonomic composition, diversity, and co-occurrence patterns of microbial communities in two distinct processing facilities: a meat processing plant (MPP) and a poultry processing plant (PPP) in the Moscow region.

2. Materials and Methods

2.1. Sampling

In this work, we studied samples of biofilms collected from the surfaces of industrial premises and technological equipment in various areas of a meat processing plant (MPP) and a poultry processing plant (PPP)

in the Moscow region. Biofilm samples were obtained by scraping surfaces with a metal spatula before the daily routine disinfection procedure (Table 1).

The scrapings were immediately placed in sterile saline solution for molecular biological and microbiological studies. The presence of biofilms in the sampled scrapings was proved by studying the structural organization using transmission electron microscopy (TEM) of ultrathin sections of the samples.

2.2. Taxonomic analysis of biofilms. DNA isolation, amplification, and sequencing of 16S rRNA gene fragments.

Total DNA from the samples was isolated using Power Soil kits (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer’s protocols. The variable V3-V4 region

Table 1. Samples taken for the study and conditions at the sampling sites

Sample No.	Zone	Sampled surface
Meat Processing Plant		
1	Carcass storage. Ambient temperature 1.8 °C	The wall of the sewer ladder (drain) in the floor
2		Condensation from the underside of the meat crate release mechanism (elevator)
3		Conveyor lubrication
4		The wall of the sewer ladder (drain) in the floor
5		Wet piece of sealant coming off the wall
6		Surface of the frame on the crate transport trolley (interior)
7		Trolley wheels
8	Accumulator for chopped raw materials	Ice with dirt from the floor
Poultry Processing Plant		
1	Evisceration zone	Ceiling
2	Poultry carcass disinfection zone	Underside of walkways near the Morris Bath
3		The ceiling above Morris’ bathroom
4	Cooling tunnel. Ambient temperature 0 °C	Wall in the carcass cooling tunnel
5		Plastic conveyor roller (top) in the cooling tunnel
6	Packaging zone	Trolley wheels (plastic) at the beginning of the packaging room
7		Trolley wheels (plastic) at the end of the packaging room

of the 16S rRNA gene was amplified using universal primers 341F (5'-CCT AYG GGD BGC WSC AG-3') and 806R (5'-GGA CTA CNV GGG THT CTA AT-3'). The resulting PCR fragments were purified using Agencourt AMPure magnetic beads (Beckman Coulter, Brea, CA, USA) and concentration was measured using Qubit dsDNA HS Assay Kits (Invitrogen, Carlsbad, CA, USA). The PCR fragments were sequenced on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) in the format of 2×300 nucleotide pair-end reads.

2.3. Bioinformatic analysis

Paired readings were combined using the FLASH v.1.2.11 program (Magoč and Salzberg, 2011). After merging, low-quality reads, singletons, and chimeras were excluded. To determine the proportion of operational taxonomic units (OTUs) in each of the samples, original reads (including low-quality and singletons) were superimposed on representative OTU sequences with a minimum identity of 97% over the entire length of the reading. To perform all these procedures, the USEARCH v.11 software package (Edgar, 2010) was used. Taxonomic identification of microorganisms by 16S rRNA gene sequences was performed using the VSEARCH v.2.14.1 algorithm in the Silva v.138 database (Rognes *et al.*, 2016). The analysis of co-presence (absence) networks was carried out based on the Sperman correlation matrix (Langfelder *et al.*, 2012) and constructed using only significant correlation coefficients (Barberán *et al.*, 2014). The threshold for correlation coefficients was set at 0.7 and the threshold for adjusted *p* values was 0.001. The analysis included only OTUs, the relative content of which was at least 5.0% in at least one sample. Alpha diversity was assessed using the Chao1 and Shannon E indices. The calculations were performed using the Usearch v11 package. Visualization and statistical analysis of diversity data were performed in QIIME. Visualization of the co-occurrence network was performed using the software package Cytoscape v.3.8.2 (Shannon *et al.* 2003; Faust & Raes, 2016). All raw data obtained of 16S rRNA gene fragments were deposited in the NCBI database and are available within the BioProject PRJNA850912 project.

2.4 Microscopic analysis of biofilms

The structural organization of material in the scrapings was studied using transmission electron microscopy (TEM) of their ultrathin sections. Each material was immediately placed in a 2.5% solution of glutaraldehyde in cacodylate buffer (0.05 M sodium cacodylate solution, pH 7.0–7.5) and kept at 4 °C for a day. Then, it was washed three times with the same buffer solution for 5 min and fixed in a solution of OsO₄ (1% OsO₄ 0.7% solution of ruthenium red in cacodylate buffer) for 1.5 h at 4 °C. After fixation, the materials were placed in 2% agar-agar and sequentially kept in a 3% solution of uranyl acetate in 30% ethanol for 4 h, then in 70% ethanol for 12 h at 4 °C. The material was dehydrated in 96% ethanol (2 times for 15 min), then in absolute acetone (3 times for 10 min). Dehydrated materials were then soaked with EPON-812 resin (Epoxy Embedding Medium Epon® 812, Sigma-Aldrich, USA) kept in a mixture of resin: acetone in a ratio of 1:1 for 1 h, then in a mixture of resin: acetone in a ratio of 2:1 in for 1 hour. The resulting material was poured into resin capsules and polymerized at a temperature of 37 °C for a day, then at 60 °C for a day. Ultrathin sections were obtained on an LKB-III microtome (LKB, Sweden) and contrasted in an aqueous solution of 3% uranyl acetate (30 min), then in an aqueous solution of 4% lead citrate (30 min). To detect acid mucopolysaccharides in biofilms, ruthenium red dye (Sigma, USA) was used, when it was added in an amount of 0.7% together with OsO₄, with which it interacted. Using ruthenium red, the presence of extracellular polysaccharides in biofilms of a number of bacteria was shown (Smirnova *et al.*, 2010). The resulting preparations were analyzed using a JEM 100SHP electron microscope (JEOL, Japan) at an accelerating voltage of 80 kV and an operating magnification of 5000–50000×. Photo documentation of the materials was performed using a Morada G2 digital optical imaging system.

3. Results

All the material scrapings obtained were evaluated for the presence of biofilms by studying the structural organization using TEM of ultrathin sections of the samples. The material scrapings were identified as biofilms when the presence of a polysaccharide matrix and a characteristic cluster of cells was visible. At the MPP, out of eight samples taken, the presence of biofilm was proven in only two samples (Table 1 and 2). Biofilms were found in sample 2, taken from the

bottom surface (with condensate) during the delivery of meat boxes to the raw material area and in sample 7, taken from the wheels of a jack-cart trolley. Of the seven samples from the PPP, six contained biofilms of varying maturity (Tables 1 and 2).

3.1 Composition of microflora of biofilms

The taxonomic composition of fifteen selected samples for MPP and PPP was determined based on the analysis of the V3-V4 variable region of the 16S rRNA gene. In total, 435,101 sequences of

variable V3-V4 fragments of the 16S rRNA gene were determined in all samples of surface biofilm contamination (Table 2). The results of clustering the obtained sequences showed the presence of 14 archaeal and 2619 bacterial OTUs with 97% sequence identity.

In the MPP, biofilms from the two locations differed in the taxonomic diversity of their microbial communities (Table 2). The condensate sample from the lower surface of the elevator for the boxes (2MPP, Chao1 — 336.8) showed lower taxonomic diversity compared to the biofilm material collected

Table 2. Chao1 and Shannon diversity indices of the studied surface biofilms obtained from the meat processing plant (MPP) and poutry processing plant (PPP)

Biofilm (refer to Table 1 for the equivalent sample locations)	Diversity indices		Number of readings (included OTUs)
	Chao1	Shannon_E	
Meat processing plant			
2MPP	336.8	3.19	65091
7MPP	697.8	4.44	42882
Poultry processing plant			
1PPP	298.6	3.82	7528
2PPP	265.3	3.80	10669
3PPP	364.8	4.05	6911
4PPP	77.1	1.68	5373
6PPP	172.3	3.32	6617
7PPP	289.8	3.77	5638

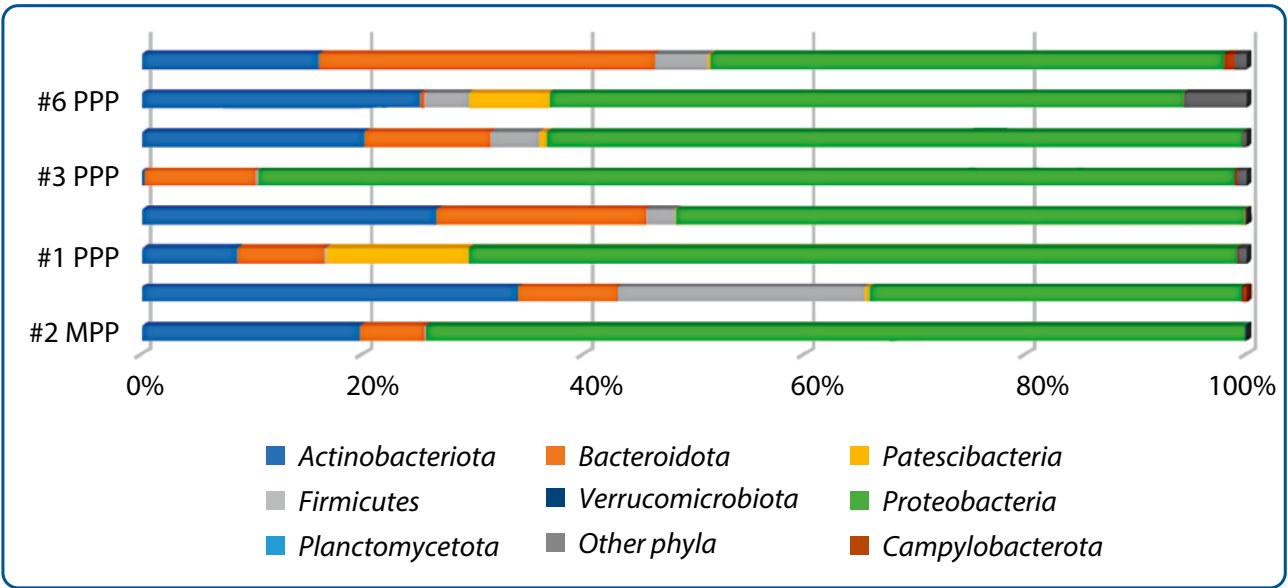


Figure 1. Distribution by class of microorganisms identified in biofilms collected from the studied meat processing plant (MPP) and poultry processing plant (PPP)

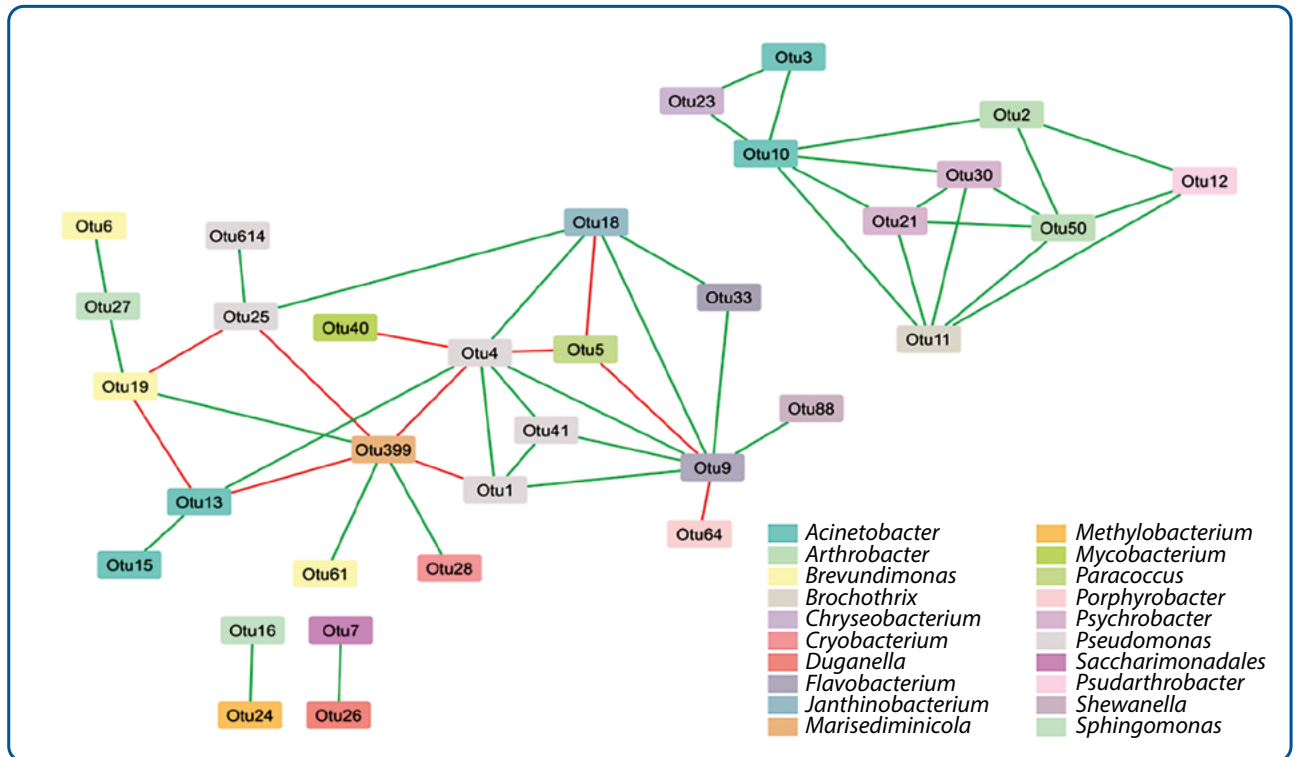


Figure 2. Network analysis of microbial interactions based on correlation analysis. Green indicates a positive interaction between operational taxonomic units (OTUs), while red indicates a mutual exclusion of OTUs.

from a trolley wheel (7MPP, Chao1 — 697.8). The taxonomic diversity of microbial communities in the biofilms from the PPP was less pronounced, as the greatest diversity was observed in biofilm 3PPP (the ceiling near the Morris bath), with a Chao1 diversity index of 364.8. The lowest taxonomic diversity was noted in biofilm 4PPP, where the Chao1 diversity index was only 77.1.

Among the bacteria detected in the biofilms, representatives of the phyla *Actinobacteriota*, *Bacteroidota*, *Proteobacteria*, *Firmicutes*, *Patescibacteria* were dominant (Figure 1).

The microbial composition of the two biofilms from the MPP differed (Figure 1). The microbial composition of biofilm 7MPP was more diverse than in biofilm 2MPP and contained a large number of classes of microorganisms. Representatives of the *Actinobacteriota* class were almost twice as numerous in the biofilm on the cart wheel (7MPP) as in biofilm 2MPP, taken from meat crate condensate. In biofilm 2MPP, most of the microbial community was occupied by a representative of the *Proteobacteria* class. At the same time, no representatives of the *Firmicutes* class were found in biofilm 2MPP, but this contrasted with biofilm 7MPP, in which they made up 22.34% of the total biofilm microorganisms detected.

The biofilms from the SPP contained representatives of *Actinobacteria*, *Bacteroidota*, *Proteobacteria*, *Firmicutes* and *Proteobacteria* in different proportions (Figure 1). The microbial community was least diverse in biofilm 3PPP (ceiling near the Morris bath), where the bulk of the microbial community was *Proteobacteria* (88.36%) (Table 2). The most diverse microbial compositions were in biofilm 6PPP (trolley wheels (plastic) at the beginning of the workshop) and biofilm 4PPP (wall in the carcass cooling tunnel).

3.2. Network analysis of interactions of microorganisms in the studied samples

Network analysis showed the largest number of positive relationships were found for representatives of the dominant genera (Figure 2). Therefore, OTU 9 (*Flavobacterium*) and OTU 10 (*Acinetobacter*) each had six possible associations with representatives of the *Psychrobacter*, *Pseudomonas*, *Brochothrix*, *Chryseobacterium*, *Arthrobacter*, and *Shewanella* genera.

The largest number of mutual exclusion relationships was found for OTU 399, belonging to the genus *Marisediminicola* (a soil bacterium) with three representatives of *Pseudomonas* (OTU 25, OTU 4, OTU 1) and one representative of *Acinetobacter* (OTU 13) (Figure 2).

4. Discussion

The study made it possible to characterize in detail the composition of microbial communities in biofilms formed on the surfaces of a MPP and a PPP. The data obtained demonstrate significant differences in taxonomic diversity and community structure between the two types of industries, which is consistent with the previously described features of microbiomes in the food industry (for example, Bokulich et al., 2016). The Chao1 and Shannon indices, reflecting alpha diversity, were significantly higher in biofilm materials from the MPP than in those from the PPP, which is likely due to more the heterogeneous environmental conditions in the MPP, including temperature fluctuations and the presence of organic substrates in the areas of cutting and storage of raw materials. On the contrary, the lower diversity in PPP can also be explained by strict temperature conditions (for example, a cooling tunnel kept at 0 °C) and regular disinfection procedures that limit the growth of some microorganisms. The dominant phyla (*Actinobacteriota*, *Bacteroidota*, *Proteobacteria*, *Firmicutes*) are typical of biofilms that form in food production plants, which is confirmed by numerous studies (Abdallah et al., 2014; Nikolaev et al., 2022; Fagerlund et al., 2021). However, unique features were observed in individual biofilms in the current study. For example, the high proportion of *Firmicutes* and *Actinobacteriota* in biofilm 7MPP (up to 22.34% and 34.03%, respectively) could be related to their resistance to drying and disinfectants, which was previously noted for industrial surfaces (Alonso et al., 2024).

DNA sequencing allowed for a detailed analysis of the microbial composition of the biofilm materials, which provides valuable information for assessing the risks associated with possible sources of contamination and the spread of pathogenic microorganisms. A key result revealed by taxonomic analysis was that biofilms at both types of meat processing plant were dominated by *Proteobacteria*, *Bacteroidota*, *Actinobacteria*, and *Firmicutes*—phyla that contain foodborne pathogens and spoilage organisms. These four phyla made up a significant part of the total microbial populations at both enterprises. It is important to note that these groups include both non-pathogenic and

potentially pathogenic species. For example, representatives of the genus *Escherichia* are classified in the phylum *Proteobacteria*, including *Escherichia coli*, one of the main causative agents of foodborne infections. The prominence of *Proteobacteria* in biofilm 2MPP (condensate) correlates with findings by Barcenilla et al. (2024), confirming that in meat processing conditions with low temperatures and high humidity, *Proteobacteria* (especially the genera *Pseudomonas*, *Acinetobacter*, and *Enterobacteriaceae*) become key components of biofilms on equipment surfaces. Notably, *Firmicutes* (which made up 22.34% of biofilm 7MPP) include the genera *Listeria* and *Staphylococcus*, which thrive on equipment surfaces (Fagerlund et al., 2017). The absence of *Firmicutes* in biofilm 2MPP could indicate localized antimicrobial interventions or nutrient limitations, warranting further investigation into site-specific microbial selection pressures.

The striking diversity disparity between the two biofilms from the MPP (Chao1: 336.8 vs. 697.8) underscores microenvironmental heterogeneity. The high diversity in biofilm 7MPP (trolley wheel) likely reflects cross-contamination from diverse sources, including soil and organic debris. In the PPP, the dominance of *Proteobacteria* (88.36%) in biofilm 3PPP (ceiling near Morris bathtub) suggests aerosolized contamination from water sources, a phenomenon documented by Elafify et al. (2024). Conversely, the low diversity in biofilm 4PPP (Chao1: 77.1) could indicate selective pressure from disinfectants, favoring resilient taxa like *Pseudomonas*.

Network analysis of microbial communities makes it possible to identify patterns of joint representation of different members of the studied communities. The network analysis conducted in the current study revealed cooperative interactions among *Flavobacterium*, *Acinetobacter*, and *Psychrobacter*—genera known for synergistic biofilm matrix production (Machado et al., 2020). These mutualistic relationships could enhance biofilm resilience, complicating eradication efforts. Conversely, the antagonism between *Marisediminicola* (soil-associated) and *Pseudomonas/Acinetobacter* suggests niche exclusion, possibly due to competition for iron or antimicrobial metabolite production (Coyte et al., 2015). However, we hypothesize that *Marisediminicola* (OTU 399) was the most unadapted (of all the organisms we detected) to the conditions of the food plants, but was not actually an antagonist of *Pseudomonas* or *Arthrobacteria*. This finding contrasts with typical biofilm synergy, highlighting the complexity of microbial interactions in industrial settings.

5. Conclusion

Based on the conducted research of microbial biofilms at meat processing and poultry processing enterprises, the following key conclusions can be drawn. Firstly, the prevalence and diversity of biofilms varied significantly between enterprises: biofilms were detected in only 2 out of 8 samples (25%) in MPP, while in 6 out of 7 samples (86%) in PPP, which indicates more favorable conditions for their formation in PPP. Secondly, microbial diversity (estimated by Chao1 and Shannon indices) was higher in MPP biofilms, especially on the surface of cart wheels (sample 7MPP, Chao1 = 697.8), which is explained by the transfer of microorganisms from soil and raw materials, while in MPP the greatest diversity was observed on the ceiling near the Morris tub (3PP, Chao1 = 364.8), and the

minimum is in the cooling tunnel (4PPP, Chao1 = 77.1) due to low temperatures (0 °C) and disinfection. Thirdly, the taxonomic composition of the dominant phylum (*Proteobacteria*, *Actinobacteriota*, *Bacteroidota*, *Firmicutes*) is consistent with typical meat industry communities, but their distribution depended on location: *Proteobacteria* (88.36%) prevailed in the condensate of the raw material elevator (2MPP), while as on trolley wheels (7MPP), *Firmicutes* (22.34%) and *Actinobacteriota* (34.03%). Fourth, a network association of microorganisms has identified a synergy between the genera *Flavobacterium*, *Acinetobacter*, and *Psychrobacter*, and an antagonism between the *Marisediminicola* and *Pseudomonas/Acinetobacter*, which highlights the complexity of inter-microbial interactions in industrial settings.

Uporedna analiza biofilmova u industriji prerade mesa i živine: taksonomija i interakcije

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INFORMACIJE O RADU

Ključne reči:

Mikrobna kontaminacija površina
Biofilmovi
Sekvenciranje visoke propusnosti

APSTRAKT

Apstrakt: Cilj ove studije bio je da se uporedi zastupljenost, taksonomska struktura i međusobne interakcije mikrobnih zajednica unutar biofilmova formiranih na površinama u pogonima za preradu mesa (MPP) i živine (PPP). Korišćenjem transmisijne elektronske mikroskopije i sekvenciranja visoke propusnosti regije V3–V4 16S rRNA gena, biofilmovi su otkriveni u 25% uzoraka iz MPP postrojenja (2/8) i u 85,7% uzoraka iz PPP postrojenja (6/7), što ukazuje na značaj uticaja faktora sredine (npr. vlažnost, higijenska praksa) na formiranje biofilmova. Taksonomska analiza pokazala je dominaciju bakterijskih koljena *Proteobacteria*, *Bacteroidota*, *Actinobacteria* i *Firmicutes* u biofilmima oba pogona, uz izraženu heterogenost u MPP uzorcima (Chao1 indeks: 336,8–697,8). Analiza mreža interakcija otkrila je kooperativne odnose između rodova *Flavobacterium*, *Acinetobacter* i *Psychrobacter*, koji doprinose otpornosti biofilmova, kao i antagonizam između bakterija iz zemljišta roda *Marisediminicola* i autohtonih *Pseudomonas*. Dobijeni rezultati naglašavaju potrebu za specifično prilagođenim higijenskim protokolima usmerenim na zone sa visokom vlagom i mobilnu opremu, kao i značaj narušavanja mikrobnih interakcija u cilju efikasne kontrole formiranja biofilmova.

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