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Review paper

Microbial biofilms in a meat processing environment

Viera Ducková[a], Miroslav Kročko[a] and Jana Tkáčová[a]*

[a] *Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia*

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ABSTRACT

The presence of pathogenic or spoilage microorganisms on surfaces in meat processing plants poses a risk of contamination of meat products by these microorganisms and the spread of foodborne diseases, as well as a reduction in the shelf life of products, even more so if these microorganisms are already attached to the surfaces in the form of biofilms. Biofilm is considered one of the major challenges for public health and food safety. The paper focuses on the characteristics of biofilms and their presence in the meat processing environment from the point of view of the representation of individual groups of microorganisms that were isolated from these biofilms during operation or after the sanitation process in meat processing plants.

1. Introduction

The consumption of meat constantly raises questions and concerns among consumers about the hygiene and safety of meat. These concerns are mostly of a biological nature and relate to the presence of pathogenic and spoilage microorganisms. In recent decades, it has become increasingly clear that bacteria including foodborne pathogens, such as *Salmonella enterica*, *Listeria monocytogenes*, *Escherichia coli*, and *Campylobacter*, together with common meat spoilage microorganisms, such as *Pseudomonas* spp., *Brochothrix thermosphacta*, *Lactobacillus* spp. and others, mostly grow in biofilms rather than in planktonic form (*Frank*, 2001; *Lindsay & von Holy*, 2006; *Sofos and Geornaras*, 2010, *Giaouris et al*., 2014; *Gaillac et al*., 2022; *Yang et al*., 2023).

The attachment of potentially pathogenic and spoilage bacteria to the surfaces of food contact machines and equipment and the subsequent formation of biofilms are serious problems for the meat industry as they can lead to cross-contamination of products, resulting in reduced shelf life and the spread of foodborne illnesses. In meat processing and meat product manufacturing environments, the microorganisms present can adhere to food contact surfaces in complex multi-species communities. Bacterial interactions are known to play a key role in the attachment and detachment of microorganism cells from biofilms, as well as in the resistance of biofilm community members to antimicrobial agents (*Sofos and Geornaras*, 2010). Disinfection of food contact surfaces in such environments is a challenging task, which is exacerbated by the high antimicrobial resistance of bacteria associated with biofilms.

This paper focuses on the characteristics of biofilms and their occurrence in meat processing environments.

***Corresponding author:** Viera Ducková, *viera.duckova@uniag.sk*

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2. Biofilm characteristics

Biofilm is not a recently discovered phenomenon, but it has been studied in the food industry for several decades. One of the first theories describing the formation of biofilms was formulated by *Costerton* in 1978, stating that most microorganisms prefer to grow as communities attached to solid surfaces. These attached bacterial populations exhibit different characteristics from their planktonic (free-swimming) forms and can exist in all nutrient-rich aquatic ecosystems, both on the body and within living organisms or on artificial surfaces (*Costerton et al.*, 1978).

There are several definitions of biofilms, with most definitions referring to biofilms as aggregations of microbial cells connected by extracellular polymeric substances (EPS or glycocalyx or extracellular matrix) that rapidly proliferate and grow on the surfaces of various materials (*Coghlan*, 1996; *Frank*, 2001; *Shi and Zhu*, 2009; *Satpathy et al.*, 2016; *Muhammad et al.*, 2020). Biofilms have been detected in various areas of food producing plants, including, e.g., floors, walls, pipes, and drains, as well as food contact surfaces and production equipment made of various materials including stainless steel, plastic, rubber, Teflon, nylon, glass, etc. (*Sofos and Geornaras*, 2010; *Wang*, 2019). The extracellular matrix of biofilms consists of polymeric compounds synthesized by microorganisms, such as extracellular polysaccharides, proteins, phospholipids or even extracellular DNA (eDNA) (*Lemon et al*, 2008; *Davies and Marques* 2009; *Rahman et al.*, 2022).

The most important characteristic of biofilms in relation to the food or meat processing environment is their increased resistance to adverse conditions (mechanical damage during sanitation, UV radiation, biocides, etc.) compared to their planktonic counterparts (*Simões et al.*, 2010; *Rahman et al.*, 2022; *Yang et al.,* 2023). This resistance is mediated by the physical barrier provided by EPS, efflux systems, differentiation of bacterial cells to an inactive state or modification of the microenvironment that may make a particular sanitizer less effective (*Giaouris et al.,* 2014; *Yang et al.,* 2023). In addition, bacteria growing in biofilms have an increased exchange of genetic information, which can result in the rapid spread of genes encoding, e.g., antibiotic resistance, between bacterial populations (*Hausner and Wuertz*, 1999; *Ch´ng et al.*, 2019; *Nikolaev et al.*, 2022a).

Another important characteristic is that biofilm microorganisms communicate with each other (*Donlan*, 2002). In the extracellular matrix of the biofilm, signal molecules can accumulate in high enough concentrations to be effective for intercellular communication and community-wide behavior (quorum sensing system) (*Sutherland*, 2001). The quorum sensing system is based on the process of autoinduction. The system provides a mechanism for self-organization and regulation of microbial cells. Bacteria excrete signals molecules (auto-inducers) into the surrounding environment, and where they accumulate during bacteria growth (*Fuqua and Greenberg*, 2002). The high cell density of microorganisms leads to an increase in the concentration of signals and induces the expression of certain genes or physiological changes in neighboring cells (*Parsek and Greenberg*, 2005). Oligopeptides and N-acylhomoserine lactones (AHLs) are the main autoinducer molecules involved in intraspecific communication in G^+ and G^- bacteria, respectively (*Fuqua and Greenberg*, 2002; *Parsek and Greenberg*, 2005). The quorum sensing system is known to be involved in a significant amount of important microbial activities. In addition to biofilm formation and synthesis of extracellular polymeric compounds, these activities include, for example, the biosynthesis of extracellular enzymes, antibiotic biosynthesis, production of biosurfactants, and extracellular virulence factors in G^- bacteria (*Daniels et al.*, 2004; *Fux et al.*, 2005). The quorum quenching system, a strategy for blocking the quorum sensing system and inhibiting the production of virulence factors, is also currently known. This strategy reduces virulence without killing pathogenic microorganisms. This system can also be called a mechanism by which bacterial communication can be interrupted, with the potential for preventing biofilm formation and to produce microbiologically safer foods. In recent years, there have been significant advances in the study of quorum sensing and quorum quenching mechanisms (*Zhang et al.*, 2019).

A microbial biofilm lives as a community of microorganisms with simple homeostasis, a simple circulatory system, and metabolic cooperation, and the response of each cell of this community is completely different from that of planktonic cells of the same species. Because it is a complex, differentiated community, its formation can be considered unique in biology, due to the coordinated activities of the relatively small genomes of prokaryotes (*Dunsmore et al.*, 1981).

3. What does it look like with biofilms in a meat processing environment?

The persistence of organic soil residues in food processing environments can lead to the formation of microbial harborages, biofilms, and niches that can serve as a source of cross-contamination (*Sofos and Geornaras*, 2010). Daily cleaning and disinfection of equipment in meat processing plants is therefore required. Sanitation is a multi-step process that aims to achieve two main objectives: a visibly clean facility (removal of food residues that support the growth of microorganisms) and a reduction of microorganism counts to an acceptable level. The goal of sanitation in food processing plants is not to achieve sterility of surfaces, and therefore, different types of microorganisms may be present on cleaned surfaces (*Langsrud et al.*, 2016; *Wang et al.*, 2018). *Jessen and Lammert* (2003) compared the effectiveness of acid and alkaline sanitation on a production line for sliced cooked ham products. Their results showed that even on visually clean surfaces after regularly performed sanitation, aerobic bacterial counts varied from < 1 CFU cm^{-2} to $3.7x10^4$ CFU cm^{-2}. These authors report that sanitization was more effective when an alkaline sanitizer with a chlorine-based disinfectant component was used compared to an acid sanitizer containing peracetic acid. Consistent with the above are the results of *Rossini and Gaylarde* (2000). On the contrary, *Fatemi and Frank* (1999) found higher efficacy with acid disinfectants composed of hydrogen peroxide and peracetic acid compared to chlorine compounds when tested in meat system. According to *Wang et al.* (2018), effective sanitation can reduce the number of indicator organisms by up to 3 log units on food contact surfaces and 1 log unit for non-food contact conveyor surfaces in a meat processing plant. The authors of this study report that the genus *Pseudomonas* was dominant among bacteria isolated from surfaces of a beef plant conveyor belt after sanitation. Among other genera, they also isolated *Comamonas, Acinetobacter, Flavobacterium, Pseudarcobacter, Bacteroides, Janthinobacterium* and *Aeromonas*. *Wagner et al.* (2020) identified ten biofilm hotspots (7 sampled during processing and 3 after sanitation) in beef, pork, and poultry meat processing plants. Five biofilms were from food contact surfaces (slicers and associated equipment and screw conveyor) and five were from non-food contact surfaces (drains and water hoses). From these biofilms, 29 different genera of bacteria were isolated. The most frequently isolated strains were from the genera *Brochothrix* (present in 80% of biofilms), *Pseudomonas* and (isolated from 70% of biofilms). The authors of this study reported that they isolated representatives of 4 to 12 different genera from each biofilm, indicating the presence of multi-species biofilms. According to *Giaouris et al.* (2014), pathogenic bacteria such as *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Campylobacter jejuni* and spoilage bacteria, e.g., *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Brochothrix thermosphacta*, *Shewanella putrefaciens*, *Lactobacillus* or *Leuconostoc*, form robust biofilms on the surfaces of food contact equipment in meat processing environments. Also, *Wang* (2019) reported that *Escherichia coli, Salmonella, Staphylococcus, Bacillus*, and *Pseudomonas* species can coexist and form biofilms in meat processing plants. In the context of pork, *Grudlewska-Buda et al.* (2023), based on biofilm analysis, pointed out that all vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* strains tested in their study showed a higher ability to form biofilms compared to susceptible strains. The taxonomic composition and structural organization of microbial biofilms at meat-processing plants (poultry, pork, and mixed materials) were also studied by *Nikolaev et al.* (2022a, b). Bacteria identified in these biofilms included *Pseudomonas, Flavobacterium, Arcobacter, Vagococcus, Chryseobacterium, Carnobacterium, Corynebacterium, Kocuria*, etc.

4. Conclusion

It can be concluded that in meat processing plants, even after the regular sanitation process, biofilm hotspots have been found, from which a wide variety of different types of microorganisms, both pathogenic and spoilage, have been isolated. It is, therefore, necessary to monitor the effectiveness of sanitation on a regular basis and at the same time look for new alternative methods (e.g., use of essential oils, bacteriophages, etc.) to minimise the spread of microorganisms. It is also important to continue to study biofilms and better understand their functioning, which may reveal new strategies for their elimination, such as the quorum quenching system.

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