Microbial Biofilm and Bacterial Contamination on Pig Carcasses

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Abstract
The aim of this study was to emphasize the presence of biofilm on meat surfaces using epifluorescences microscopy and establishing the microbial contamination level by classical microbiological methods. The research was performed in a pork slaughterhouse. The presence of microbial biofilm and the level of contamination were performed on surfaces from pig carcasses and cut pieces. Clusters of microorganisms included in a biofilm matrix were found on the surface of carcasses on sternal region, coast region, coccigian region and on surfaces of cut pieces: chop, front of thighs. Microbial biofilm was present on carcasses and cut pieces at least 3 days length, in regions with high humidity and microbial contamination level ranged of 10^2- 10^3 cfu/ cm^2. The microbial load of the surfaces was assessed using the following microbiological indicators: *total viable count (TVC),* the *number of enterobacteria* and *Pseudomonas* genus. The level of carcasses contamination ranged on average from 1.3 x 10 cfu/ cm^2 (neck) to 2.6 x 10^3 cfu/cm^2 (front of pulp). The proportion of *Enterobacteriaceae*-positive samples was 60%, with a low level of contamination (less than 1 cfu/ cm^2). Germs of the *Pseudomonas* genus were absent in all the analyzed samples.

Keywords: biofilm, microbial contamination, pig carcass

1. Introduction

In slaughterhouses and processing plants the surfaces of carcasses and fresh meat are easily contaminated with microorganisms that come from a variety of sources (hides, feces, air, water, equipment, utensils, humans, etc.) and consist their growth support if the handling, processing and preserving are not proper.
Contamination with spoilage microorganisms may lead to product and economic losses, while presence of pathogens or their toxins may be the cause of foodborne disease [1].[1]
Some microorganisms that contaminate the meat surface manage to survive, multiply and even to form complex structures like microbial biofilms, which is a form of adaptation to poor environmental conditions (temperature, humidity, pH). The ability to form biofilm was emphasized in many types of microorganisms involved in hygiene and food safety (*Escherichia*, *Listeria*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Aeromonas*, *Aerobacter*, *Acinetobacter* etc..), yeast (*Candida albicans*) [2].
The study of attached microorganisms and biofilm formation on various surfaces by classical microbiological methods provides guidance just on the presence of viable microorganisms, but doesn't provide information about the biofilm structure and does not detect harmed microorganisms, such as viable but non cultivable microorganisms.
Identifying the microbial biofilm formation can lead the processors to improve the sanitation and processing technologies.
The aim of this study was to emphasize the presence of microbial biofilm on meat surfaces using epifluorescences microscopy and to establish the level of contamination by classical microbiological methods

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2. Materials and methods

The research was performed in a pork slaughterhouse. The presence of microbial biofilm and level of contamination were performed on refrigerated carcasses and cut pieces surfaces.
Samples were collected from the following regions: masseter muscle, neck, chest, sternum, the internal coast region, axillary, ventral abdominal, lumbar, caudal, front of thighs, chop. Sampling was performed by rubbing, using sterile swabs immersed in physiological peptone solution, from a surface of 100 cm^2. The samples were transported to the laboratory in refrigerated conditions.
To identify the biofilm, samples were taken by scrapping the surfaces with a knife. For each point of sampling we took two samples in a similar manner. The first series of slides were stained *Gram* and the second series with acrydine orange following the technique describes below: the samples were fixed on the slide with ethanol 96% for two minutes; the dehydrated samples were treated with Hanks modified solution (without D glucose and phenol red) and fixed with ethanol 96% for two minutes. The samples were stained with acrydine orange for one minute [3]. The samples were analyzed by using an epifluorescent microscope model *Leica DM 2500* with external UV light source *Leica EL 6000*.
The level of contamination of the surfaces was assessed using the following microbiological indicators: *total number of viable coun*t, the *number of enterobacteria* and the *number of germs from Pseudomonas* genus.
The sampling was performed from the same region of the carcasses as the sampling made for biofilm identification.
Total number of germs determination was made according to SR ISO 4833/ 2003 [4]. The test tubes were shaken and 1 cm^3 of physiological solution was drawn and inoculated in two *Petri* dishes and then pour PCA culture medium and mix together. After 72 hours of incubation at 30°C the counting of colonies was made.
The *enterobacteria number determination* was made according to SR ISO 21528-2/ 2007 [5]. The enterobacteria isolation was made on *VRBG* medium (violet, red, bile, glucose agar). After 24 hours at 37° C the pink, red or purple colonies were counted selected and five typical colonies were tested for oxidase production and glucose fermentation.
The *pseudomonas germs determination* was made on the special medium for pigment production identification.

3. Results and discussion

Microbial biofilm on pig carcasses
Microscopic analysis of the samples taken from pig carcasses and cut pieces surfaces revealed the presence of isolated microorganisms and clusters of microorganisms included in a matrix.
Clusters of microorganisms included in a biofilm matrix were found on the surface of carcasses from sternal region (Figure 1), coast region (Figure 2) coccigian region and on cut pieces: chop, front of thighs (Figure 3, Figure 4).

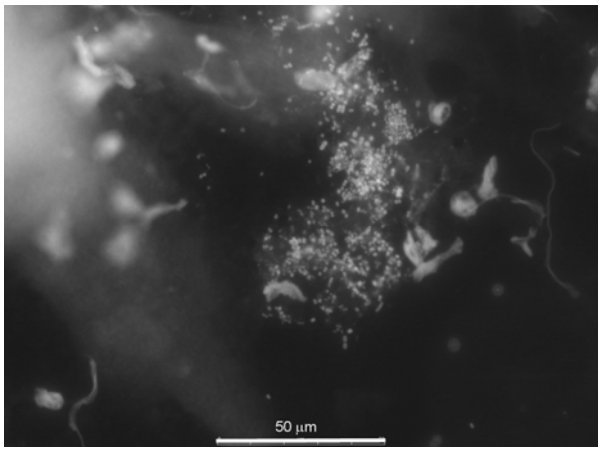

Figure 1. Microbial biofilm on sternal region

Microbial biofilm was present on carcasses and cut parts with a length of at least 3 days.

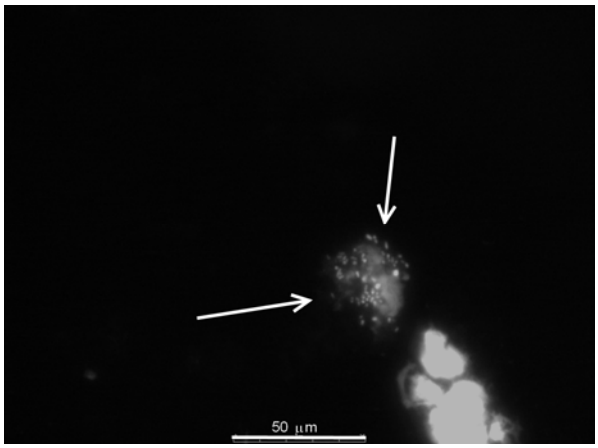

Figure 2. Microbial biofilm on coast region

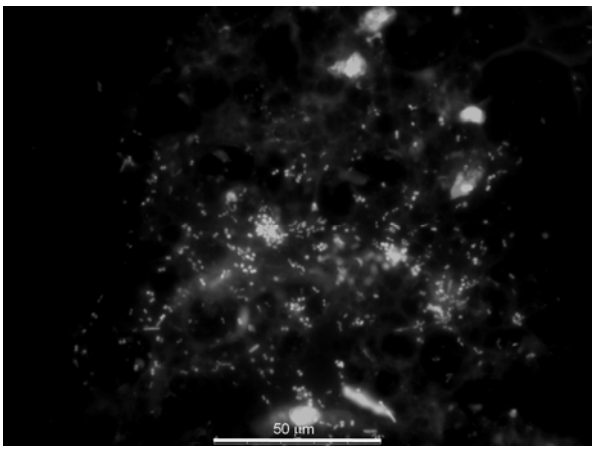

Figure 3. Microbial biofilm on chop

These surfaces had high humidity.
Following the samples Gram stain examination from the same points were the microbial biofilm was present, in all samples Gram positive cocci and Gram negative bacilli were isolated.

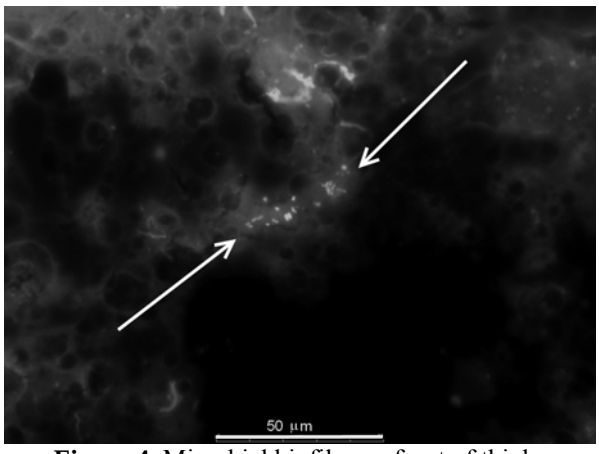

Figure 4. Microbial biofilm on front of thighs

On the surfaces where the biofilm was present the level of microbial contamination ranged on average from 10^2 to10^3 cfu/cm^3, enterobacteria were present in small numbers, and germs from *Pseudomonas* genus were absent.

The level of microbial contamination on pig carcasses

The contamination of carcass surface is performed in different phases of processing, through contact with dirt from hoofs, content of digestive tract, working surfaces processing unit, equipment and hands of worker, the water used to wash the carcasses, the air of working area. Any operation of cutting, processing, storage, transport and distribution are additional opportunities for further contamination. The level of contamination reflects the hygiene conditions in slaughterhouse and the composition of microbial flora reflects the source of contamination and the effectiveness of preventing the meat contamination.

There is a close correlation between the initial number of microorganisms that contaminate the meat and the occurrence of spoilage. The highest the level of contamination the faster is the spoilage of the meat. In hygienic cooling chambers the air can contribute to increase the number of microorganisms present on the carcasses with 14 cfu/ cm^2/ day [6].

The level of carcasses contamination ranged on average from 1.3 x10 cfu/ cm^2 (1.13 log cfu/ cm^2) to 2.6 x 103 cfu/ cm^2 (3.42 log cfu/ cm^2). The highest level of contamination was found in samples from the front of pulp (2.6 x 10^3 cfu/ cm^2). The increased level of microbial contamination can be explained by the fact that this region is more exposed to contact with the external surface or other areas. Furthermore, the multiplication of microorganisms is favored by high humidity at this level, resulting from continuous diffusion of water from deeper layers of meat to the surface, a phenomenon that occurs if the relative humidity of storage space is under 95%. The lowest level of contamination was found in samples from the neck (1.3 x 10 cfu/ cm^2) that is likely to be low due to differences in structure of the meat at this level, mainly fatty tissue. *Bărzoi and col.* [6], in flesh covered with fat the water diffusion is stopped, and the development of microorganisms is lower.

The results are similar to those obtained by *Zweifel and col.* [7], who found fluctuations in microbial level of contamination between 2.2 and 3.70 log cfu/ cm^2, in a study conducted in five slaughterhouses, on 650 pig carcasses.

The proportion of *Enterobacteriaceae*-positive samples was 60%, but the level of contamination was very low (less than 1 cfu/ cm^2). Knowing that enterobacteria are indicators of fecal contamination, their detection in small number reflect the high degree of hygiene on the technological flow, rapid cooling in a short period of time after obtaining, the microclimate conditions in storage areas for carcasses, etc. The results are comparable with those obtained in other research. For example, *O 'Brien and col*. [8] have identified *Enterobacteriaceae* in 76% of samples, and *Hutchinson and col.* [9] in 56% of samples taken from the pig carcasses surfaces.

It is surprising that germs of the *Pseudomonas* genus were absent in all samples analyzed. It is known that on chilled carcasses, psihrotrofe aerobic bacteria species predominate, represented by *Pseudomonas, Acinetobacter, Moraxella*.
With regard to performance criteria of *Regulation (EC) No 1441/2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs* [10], all results of *TVC*s and *Enterobacteriaceae* from pigs carcasses was rated as satisfactory (below 4 log cfu/ cm^2 for TVCs and 2 log cfu/ cm^2 for *Enterobacteriaceae*, respectively).

4. Conclusions

The microbial biofilm is present and can be emphasize on the surface of pig carcasses and cut parts that have a length of at least 3 days.
Microbial biofilm can be found in regions with humidity and microbial contamination level ranged on average from 10^2 to 10^3 cfu/ cm^2.

Acknowledgements

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