The role of monitoring production environment facilities to support microbiological safety and food quality in meat processing plants

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Abstract. The results of many studies prove the microbiota of the surfaces of the production environment can be a source of food contamination. Environmental monitoring allows to identify problem areas in the enterprise and take corrective actions to eliminate them. This work is conducted to the analysis of the microbiota of abiotic objects selected in the area of close proximity to food products at a pork processing plant by sequencing the 16S RNA gene. The phylum *Proteobacteria* (from 37.7 to 73.6%), *Firmicutes* (from 0.14 to 18.6%), *Bacteroidota* and *Actinobacteriota* were the dominant components of the microbial communities of the meat processing enterprise. Bacteria of the genus *Pseudomonas* were found in all samples, the number of readings of these bacteria ranged from 1.90% to 28.76% of the total number of readings. Bacteria of the genus *Brochotrix* were found in samples from 0.02% to 2.75%. The identification of this phylum indicates the potential presence of pathogenic microorganisms and spoilage microorganisms at production facilities, which can negatively affect the quality and safety of food products.

1 Introduction

Food safety and quality can be compromised by microbiological contamination caused by various microorganisms present in the production environment. As a rule, they enter the food environment through raw materials, pests, air, water and employees. Usually, the routine application of appropriate sanitary standards makes it possible to control these microorganisms in food processing conditions. However, if the level of contamination is high or sanitary procedures are ineffective, microorganisms can gain a foothold, for example, in biofilms and contaminate food products, which will lead to outbreaks of infectious diseases of food origin. Therefore, it is extremely important to monitor the microbiological status of the objects of the production environment at food production enterprises. products [1]. The Environmental Monitoring Program (EMP) evaluates the effectiveness of general hygiene procedures at the enterprise and provides the necessary information to prevent possible microbial contamination of food products [1]. Sampling can

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be carried out before, in the middle or after a change in the food process in accordance with the purpose of the EMP. If the purpose is to verify the effectiveness of sanitary measures, then sampling should be carried out after the cycle of sanitary work and before production. If contamination is suspected at the time of production of products, for example, from equipment, sampling can be carried out during operation of the equipment [2].

An effective microbiological environmental monitoring program will provide early warning of potential microbiological hazards in a food enterprise, identify problems, provide scientific data for source investigation and provide general microbiological control [3]. The work includes microbiological sampling of flushes from equipment, tools, surfaces, personnel and premises to identify pathogens and spoilage microorganisms of concern [3].

The importance of studying the microflora circulating in food enterprises as a reservoir of microbial contamination has been emphasized by outbreaks of food origin in the past [4-5]. Due to the correct approaches to microbiological environmental monitoring, the food industry can evaluate the effectiveness of sanitary methods, identify areas of improvement and prevent the presence of pathogens, allergens or chemical pollutants in food products.
The purpose of this work was to assess the microbiological status of industrial environment objects located in close proximity to food products and identify the main groups of microorganisms as a possible source of food contamination.

2 Experimental materials and methods

The objects of the study were 11 samples from environmental facilities at a meat processing plant in 2022. For evaluation, sampling was carried out in the course of work. The identification of the sampling sites is shown in Table 1. The samples for the study were selected from the area of close proximity to food products.

Table 1. Sampling areas.

No.	Laboratory number	Objects
A66	No.1	The tiled wall
A67	No.2	The wall opposite the conveyor with carcasses
A68	No.3	Container washer
A69	No.4	Lubricant from the carcasses chain
A70	No.5	Sawing control panel
A71	No.6	Conveyor of clean containers
A72	No.7	Pallet rack
A73	No.8	Finished product line
A74	No.9	Wall in the storage room
A75	No.10	Vacuum machine
A76	No.11	Band saw

2.1 DNA isolation, amplification, and sequencing of 16S rRNA gene fragments

Isolation of total DNA was performed using a modified Birnboim-Doly alkaline isolation procedure and Wizard technology from Promega. DNA concentration was measured on a spectrophotometer SmartSpec 3000 (BioRad, USA). Determination of the nucleotide sequence of the total amplification of the 16S rRNA gene fragments (V3-V4 region) was carried out by high-throughput sequencing on the platform MiSeq (Illumina, США). The resulting library was sequenced on MiSeq (Illumina, San Diego, CA, USA) using Miseq Reagent Kit V3 in the format of 2×300 nucleotide pair-end reads.

2.3. Bioinformatics analysis

Paired readings were combined using the FLASH v.1.2.11 program. After merging, low-quality reads, singletons, and chimeras were excluded. The remaining readings were clustered into operational taxonomic units (OTUs) with at least 97% identity. To determine the proportion of 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. 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.

3 Results

The taxonomic composition of eleven samples was determined based on the analysis of the V3-V4 variable region of the 16S rRNA gene. A total of 5,238 sequences of variable V3-V4 fragments of the 16S rRNA gene were determined in all surface contamination samples (Table 2).

The smallest taxonomic diversity of microbial communities was observed in the sample of grease from the line of removing half–carcasses from hooks (A69, Chao1 -262.8), and the greatest taxonomic diversity was observed in the sample of flushing from the wall in the storage hopper for semi-finished products (A74, Chao1 - 638.4). The Shannon Index is an indicator of the diversity of species (types) of living beings in an ecosystem, which is based on a weighted average geometric value of the proportional share of a species in a community. The index takes into account both the total number of species and the heterogeneity of their representation in the ecosystem. The greatest diversity of microbial communities according to the Shannon index (shannon_e) was noted in the sample of flushing from a vacuum machine in the packaging shop of large-batch semi–finished products (A75, shannon_e - 5.12), and the lowest value in the sample of lubrication from the line of removing half-carcasses from hooks (A69, shannon_e – 2.36).

Table 2. Assessment of the diversity of microbial communities.

Sample number	Number of OTE	chao1	shannon_e	Completeness, %
A66	330	332.3	3.93	99.31
A67	570	571.2	3.97	99.79
A68	506	507.1	3.46	99.78
A69	262	262.8	2.36	99.70
A70	402	403	4.33	99.75
A71	380	381.2	3.11	99.69
A72	525	525.7	3.93	99.87
A73	563	564.5	3.97	99.73
A74	637	638.4	4.17	99.78
A75	593	594.3	5.12	99.78
A76	470	471.1	4.12	99.77

In all cases, the completeness of diversity detection was more than 98%. Thus, the data obtained allow us to describe the composition of the studied communities quite fully.

The taxonomic classification of the obtained OTE was carried out using the Silva 16S rRNA sequence database [6]. The results of the taxonomic analysis of the composition of microbial communities based on 16S rRNA gene sequences at the phylum level are presented in Figure 1.

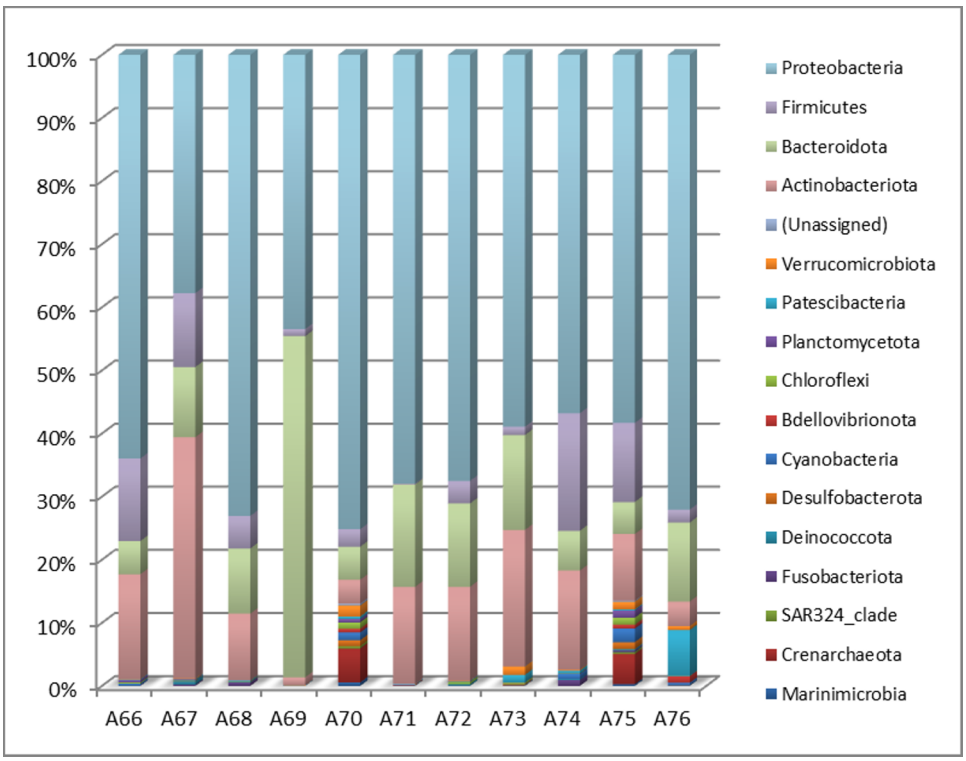

Fig. 1. Taxonomic diversity of samples at the phylum level.

In the microbiota of production facilities at food processing enterprises, the diversity of microbial communities was represented by more than 40 phylum's. Representatives of four phylum dominated: *Proteobacteria, Firmicutes, Bacteroidetes* and *Actinobacteriota*. The phylum *Proteobacteria* turned out to be the most abundant component of microbial communities of objects in the zone of close proximity to food products at a pork processing plant and ranged from 37.7 to 73.6%. The frequency of occurrence of 16S rRNA gene copies of representatives of the phylum *Firmicutes* ranged from 0.14 to 18.6%.
Figure 2 shows the taxonomic composition of the main phylum at the family level.

The phylum Actinobacteriota was represented by seven main families, among which Micrococcaceae dominated from 0.55% from the flushing from the sawing control panel (next to the circular saws, cut into cuts) to 19.83% in the sample flushing from the wall of the raw materials workshop (opposite the conveyor with half-carcasses). The phylum *Bacteroidota* is represented by 11 families, the predominant number of representatives of the families *Nocardioidaceae, Dermabacteraceae, Sphingobacteriaceae* and *Weeksellaceae*. The phylum *Firmicutes* was represented by 11 families. Representatives of the *Bacillaceae* families were the most common, *Carnobacteriaceae, Staphylococcaceae*, *Streptococcaceae, Listeriaceae*. The greatest diversity of families was observed within the phylum *Proteobacteria* and was represented by 37 families. Representatives of the families *Beijerinckiaceae, Comamonadaceae, Moraxellaceae, Oxalobacteraceae, Pseudomonadaceae, Rhodobacteraceae, Sphingomonadaceae, Xanthomonada*ceae were most often found in most of the studied samples.

In addition to potentially pathogenic bacteria, bacteria of the genera *Brochothrix* and *Pseudomonas*, which cause spoilage of meat and meat products, were detected in the samples. Bacteria of the genus *Brochothrix* were found in all samples, with the exception of A69, the number of readings of these bacteria ranged from 0.02% to 2.75% of the total

number of readings. The largest proportion of bacteria of the genus *Brochothrix* was observed in samples A68 (2.7%) and A74 (2.1%). Bacteria of the genus *Pseudomonas* were found in all samples, the number of readings of these bacteria ranged from 1.90% to 28.76% of the total number of readings. The largest proportion of *Pseudomonas* bacteria was observed in samples A74 (28.8%), A69 (27.8%) and A70 (22.8%).

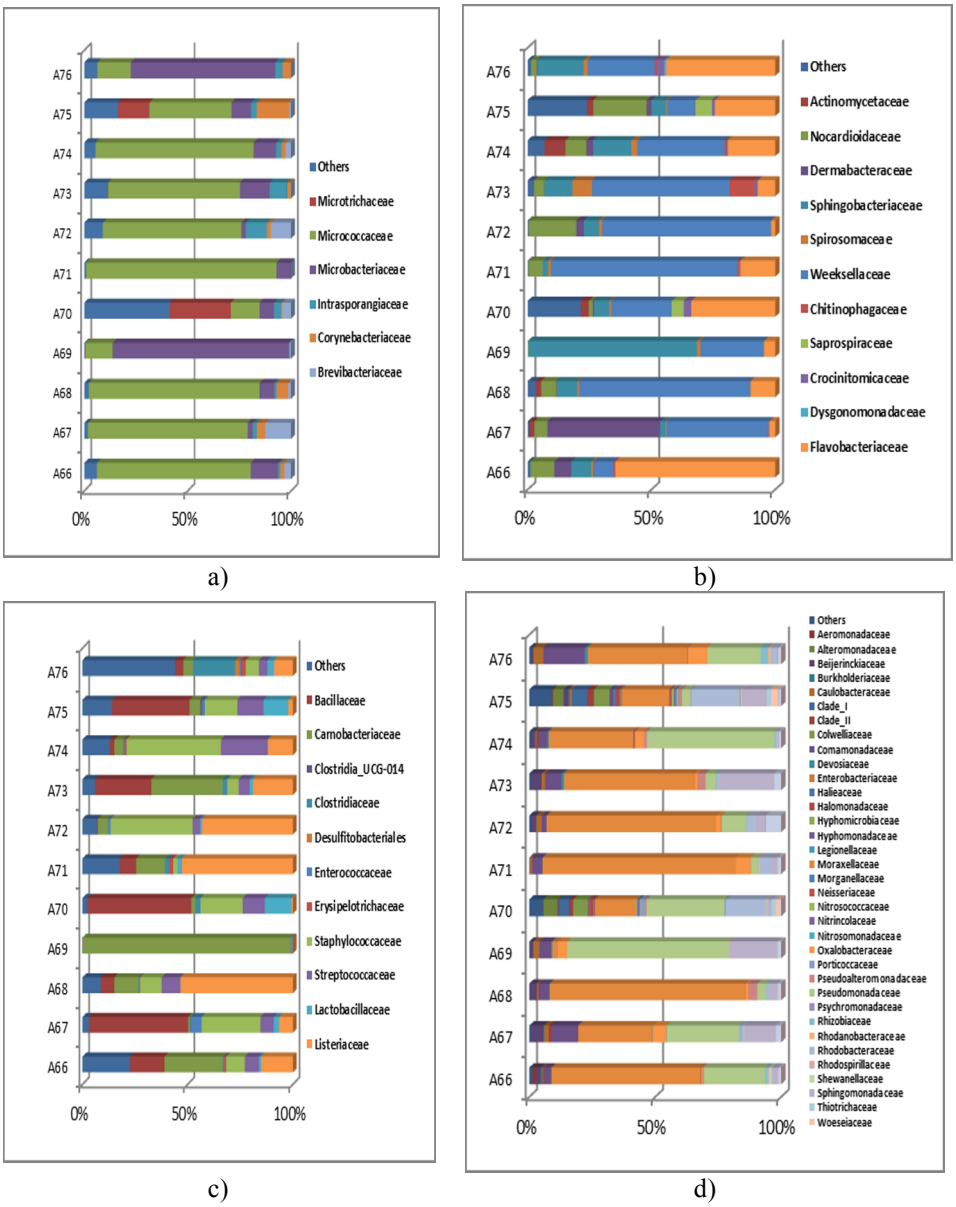

Fig. 2. Taxonomic diversity of specimens at the family level from a) *Actinobacteriota*; b) *Bacteroidota;* c) *Firmicutes;* d) *Proteobacteria.*

4 Discussions and conclusion

In the course of our work, when evaluating the microbial community of a pork processing enterprise, it was revealed that representatives of four phylum dominated on the surfaces of abiotic objects in the area of close proximity to food products: *Proteobacteria* (from 37.7 to 73.6%), *Firmicutes* (from 0.14 to 18.6%), *Bacteroidota* and *Actinobacteriota*. The detection of these phylum indicates the potential presence of bacteria such as Salmonella spp., *Listeria* sp., *E. coli*, *Yersinia* sp. at production facilities. In a study on the assessment of the microbiome of a processing plant for the production of boiled sausages, a phylogenetically close to the species *Yersinia pseudotuberculosis* was found. At the same time, yersine-like OTE was found in 28% of surface samples of processing plants and only in <1% of samples of raw meat, emulsions and sausages [7]. It is also worth noting that yersine-like OTE were found on the surfaces of the packaging room, which assumes high hygienic requirements. This fact raises concerns related to the persistent existence of pathogenic bacteria on surfaces and environmental objects.

The probability of the presence of *Listeria* spp., including *L. monocytogenes*, is indicated by the high frequency of occurrence of a copy of the 16S rRNA genes of representatives of the phylum *Firmicutes* (from 0.14 to 18.6%). There is evidence that resistant *L. monocytogenes* is difficult to combat, since they are present in hard-to-reach places inside a room or equipment that may not be easy to clean and disinfect [8]. Such "niche" places were chosen by us to study the microbiota of surfaces. In addition to pathogenic bacteria, among the identified representatives of microbial communities of surfaces, spoilage bacteria were found: *Brochotrix* sp. and *Pseudomonas* sp. Their predominance on surfaces among other species may be due to the ability to form and exist as part of mono- and multi-biofilms. This is confirmed by the work of Austrian scientists who investigated the generic microbial composition of biofilms found at a meat processing plant [9]. The most common bacteria were microorganisms of the genera *Brochothrix* (present in 80% of biofilms), *Pseudomonas* and *Psychrobacter* (isolated from 70% of biofilms), including in places in close proximity to food.

A group of scientists from Australia analyzed the microbiota of local cattle slaughterhouses. The results of the assessment of the microbial diversity of the surfaces of hides, carcasses and objects were the predominance of such phylum as *Lactobacillales* (2.4–56.2%) and *Pseudomonadales* (2.4–59.4%) in slaughterhouse B and *Bacteroidales* (3.9–43.8%), *Lactobacillales* (0.0–61.9%) and *Pseudomonadales* (0.5–72.1%) – in slaughterhouse A [10]. Such a difference in the dominant microflora of environmental objects may be due to the fact that the composition of microbial communities depends on many factors: location, seasonality, type of enterprise and its hygienic status [11]. In recent years, many studies have been conducted, the results of which prove that the microbiota of the surfaces of the production environment can be a source of contamination of the finished product. Therefore, knowledge about the circulating microflora is necessary for each individual enterprise, conducting microbiological monitoring on an ongoing basis will not only establish the microbiological status of production facilities, but also prevent the spread of pathogens and spoilage microorganisms.

Environmental monitoring plays a crucial role in the food industry to ensure food safety and quality.

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