Original scientific paper

Composition and diversity of microbial communities of industrial environment objects

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A B S T R A C T

One of the main sources of bacteria that cause spoilage is microorganisms harbored on surfaces and objects in the production environment of food enterprises. This paper presents the results of the taxonomic composition of the microbiota of objects in the production environment of pork processing enterprises, with the identification of key groups of microorganisms. The microbiota in the facilities of the production environment of the meat processing enterprise was mainly represented by 12 phyla. Among these, Proteobacteria, Bacteroidota, Actinobacteria, and Firmicutes were predominant. The results of 16S rRNA amplicon sequencing were analyzed for the presence of pathogenic bacteria in the studied samples. Potentially pathogenic *Shigella* bacteria were found in two samples. In addition to pathogenic bacteria, bacteria detected in the samples cause spoilage of meat and meat products; these were the genera *Brochothrix* and *Pseudomonas*. Pathogenic microorganisms were studied by the molecular method with accumulation. Representatives of pathogenic microorganisms were present on the objects in the production environment. *Listeria monocytogenes* was found in three samples, *Salmonella* spp. in two samples, and *Campylobacter* spp. in one of the samples studied.

1. Introduction

The food industry is developing with serious complications concerning reduced susceptibility to foodborne pathogenic bacteria during lapses (*EFSA and ECDC,* 2016). One of the main sources of food spoilage are microorganisms from objects in the production environment in food enterprises, which was identified in previously (*Stellato et al.,* 2016).

Although this is a global economic and health problem, until recently, little was known about microbial diversity in slaughterhouses and meat processing plants, and it was difficult to track and control ways to reduce the risk of meat spoilage.

Moreover, certain taxa or strains with good biofilm-forming capacity are able to survive in sanitary conditions and/or increase adaptive responses to stress. The meat industry continues to rely on standard microbiological methods for hygienic production control. Such methods can be useful in determining the general condition of an object, but they do not allow us to describe the complex microbial communities formed within a particular production complex (*Zwirzitz et al.,* 2020).

In the last decade, attempts have been made to characterize the microbiome in the food industry using molecular genetic methods (*Bokulich et*

al., 2015*; Yang et al,* 2016). Techniques such as next generation sequencing (NGS) allow the detection of microorganisms in food without prior culture and without isolation of species-specific fragments from total DNA and amplification of genes encoding rRNA (*Ercolini*, 2013*; Lozupone and Knight,* 2005). In addition, it allows the creation of object-specific bacterial flux maps that show unique transmission patterns for individual taxa. This approach helps to increase knowledge about the transmission of microorganisms, thereby improving hygiene standards in the food industry to improve food safety.

The aim of the work was to study the taxonomic composition of microbiota on the production environment surfaces and objects in a pork processing enterprise, with the identification of key groups of microorganisms.

2. Materials and methods

2.1 Research subjects

The objects of the study were 36 samples taken in September 2020 from production surfaces and equipment at a meat processing enterprise (21 samples). The list of analyzed samples is presented in Table 1.

2.2 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. (США) (*Bulygina et al.,* 2002). DNA concentration was measured on a spectrophotometer Smart-

Table 1. Environmental samples taken in the meat processing plant

Number	Zone	Point
A1		sorting table 1
A2		conveyor belt 1
A3		equipment case
A4		cleaning equipment (hose)
A5		sorting table 2
A6		conveyor belt for carcasses 2
A7		work aprons
A8		cleaning equipment (mop)
A9	Preparation for cutting and deboning	equipment maintenance tool
A10		by-product conveyor belt
A11		conveyor belt 3
A12		work gloves
A13		waste bin (external part)
A14		carcass (external part)
A15		carcass (internal part)
A16		knives for cutting
A17		plastic container
A18	Chilling	wall
A19		carcass cutting saw
A20	Cutting and deboning	table
A21		hooks for pork heads

Spec 3000 (BioRad, USA). The DNA concentration in the preparations ranged from 10 to 40 ng/µl, whereby A260/A280 = 1.8−1.9. 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 variable V3-V4 region of the 16S rRNA gene was amplified using universal primers 341F (5'-CCTAYG GGDB-GCWSCAG) and 806R (5'-GGA CTA CNVGGG THTCTAAT) (*Frey et al.*, 2016). 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 (*Magoč and Salzberg*, 2011). 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. 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).

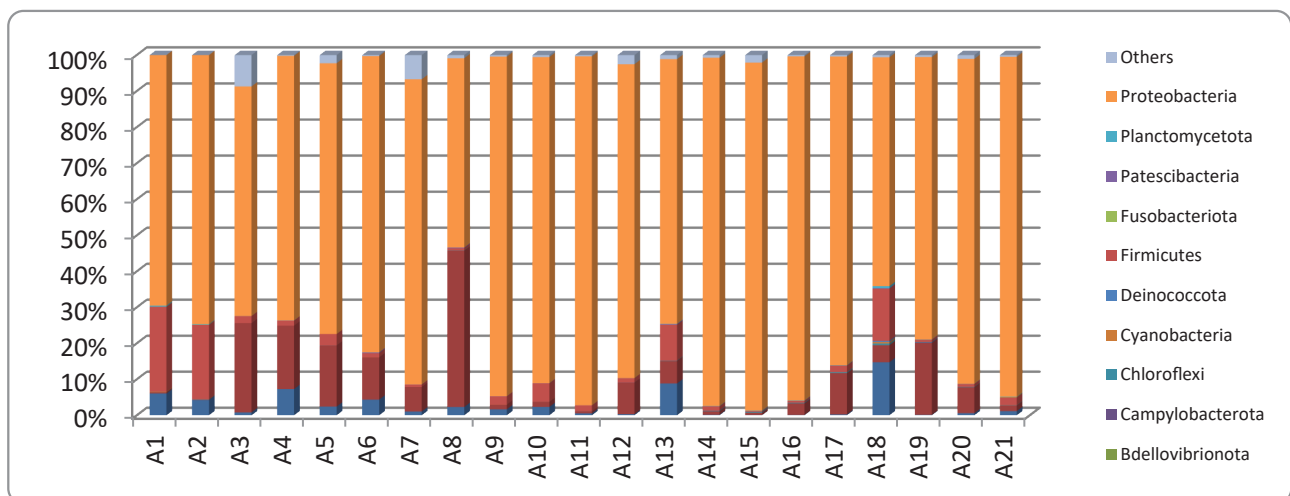## 2.4 Detection of pathogenic bacteria

The detection of *Listeria monocytogenes, Salmonella* spp.*,* and *Campylobacter* spp. was conducted by the commercial LAMP-based kit (3M Molecular Detection Assay Listeria monocytogenes; 3M — for Listeria monocytogenes detection; 3M Molecular Detection Assay Salmonella — for Salmonella spp. detection; 3M Molecular Detection Assay Listeria monocytogenes — for Listeria monocytogenes detection), used according to the manufacturer's manual. All samples identified as positive by molecular analysis were confirmed by standard laboratory tests in accordance with Russian Standard Methods. Confirmation of *L. monocytogenes* was performed according ISO 11290. Confirmation of *Campylobacter* spp. was performed according to GOST 10272 part 1. Confirmation of *Salmonella* prevalence was performed according the Russian Standard method for the detection of *Salmonella* spp. GOST 31659.

3. Results

After processing the sequencing data, the sequences for all accessions were pooled into a cluster of 4020 operational taxonomic units with a minimum identity of 0.97.

The taxonomic classification of the obtained OTUs was carried out according to the Silva 16S rRNA sequence database (*Quast et al., 2013*). The results of the taxonomic analysis of the composition of microbial communities according to the 16S rRNA gene sequence are shown in Figure 1.

The microbiota on the production environment surfaces and objects in the meat processing enter-

Figure 1. Taxonomic composition of microbial communities of samples taken from the meat processing plant A1-A21

Table 2. The results of sequencing of 16S rRNA amplicons for the presence of pathogenic bacteria

Bacteria	Number OTU identity	OTU
Salmonella	Missing	-
Listeria monocytogenes	Missing	-
Shigella	A1-0.02 %, A11- 0.02%	Otu4
Brucella	Missing	-
Campylobacter jejuni	Missing	-
Clostridium perfringens	Missing	-
Staphylococcus aureus	Missing	-

prise was mainly represented by 12 phyla. Among these, Proteobacteria, Bacteroidota, Actinobacteria and Firmicutes were predominant. Proteobacteria dominated in samples A9, A11, A14, A15, and A16, in which they accounted for more than 90% of the data obtained. Sample A8 contained representatives of the phylum Bacteroidota to the greatest extent (43.29%). Slightly fewer Bacteroidota were present in samples A3, A4, A5, A6, and A19, where the percentage ranged from 11.62% to 24.79% depending on the sample. Firmicutes were found in all studied samples; however, their maximum percentage was noted in samples A1 and A1, at 23.20% and 20.39%, respectively. Actinobacteria were presented in various amounts in the samples, and the maximum percentage of this phylum, 14.64%, was in A18. Minor groups included representatives of Bdellovibrionota, Campylobacterota, Chloroflexi, and Fusobacteria.

The results of sequencing of 16S rRNA amplicons were analyzed for the presence of pathogenic bacteria in the studied samples. At the same time, potentially pathogenic *Shigella* were found in samples A1 and A11 (Table 2). However, the number of *Shigella* 16S rRNA reads in the samples did not exceed 0.02% of the total number of reads.

In addition to 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 samples A3, A4, A5, A7-A10, A12, A13, A16-F18. The number of readings of these bacteria ranged from 0.01% to 6.14% of the total number of readings. Bacteria of the genus *Pseudomonas* were found in all samples; the number of readings of these bacteria ranged from 0.19% to 78.47% of the total number of readings. The largest proportions of bacteria of the genus *Pseudomonas* were observed in samples A21 (78.47%), A20 (69.95%), A14 (67.81%), A13 (66.85%), A9 (64.72%) and A11 (61.68%).

Pathogenic microorganisms were investigated by the molecular method with accumulation. Pathogenic bacteria were present on the production environment surfaces. *L. monocytogenes* were found in three samples A2, A11, and A17 (Table 3).

Table 3. Pathogenic bacteria in environmental samples

Bacteria	Sample number	Point
Listeria monocytogenes	A2	conveyor belt 1
	A11	conveyor belt 3
	A17	plastic container
Salmonella spp.	A2	conveyor belt 1
	A5	sorting table 2
Campylobacter spp.	A19	carcass cutting saw

Samples A2 and A5 were contaminated with *Salmonella*. One of the natural samples of A19 contained *Campylobacter* spp.

4. Discussion

During the analysis of the microbiome, the main phyla of the meat processing enterprise were Proteobacteria and Firmicutes. It is known that these microorganisms are representatives of the intestinal microflora of pigs and are often found on the surface of biotic and abiotic objects of meat processing enterprises (*Zhang et al.,* 2020). The distribution of these phyla on the surface of carcasses is interesting: there is evidence that Proteobacteria are more often found on the surface of the upper part of the carcass, and Firmicutes on the surface of the lower part. This is probably due to the characteristics of primary processing and surface contamination in one way or another (*Steven et al.,* 2022*; Braley et al.,* 2022). Bacteria of the genus *Pseudomonas* were found in all samples, and the number of readings of these bacteria ranged from 0.1% to 43.6% of the total number of readings (*Yu et al*., 2020). This is confirmed in scientific works of recent years, where *Pseudomonas* is a significant part of the microbial community of abiotic objects in meat processing enterprises (*Cobo-Díaz et al.,* 2021). In a study on shelf life, *Chen et al. (*2020) did not find *Pseudomonas* on chilled poultry carcasses, but found them on the walls of the air cooler, and by the end of 12 days of storage, they dominated the microbiota of packaged carcasses (*Chen et al,* 2020). This once again proves the influence of the bacterial status of production environment objects on the microbiota of products during processing and storage. During our study, the genus *Shigella* was found, the reservoir for which is also the gastrointestinal tract. How-ever, the number of *Shigella* 16S rRNA reads in the samples did not exceed 0.02%. This suggests that indicator microorganisms determined by classical microbiological methods (for example, fecal contamination — *E. coli*) may represent only a fraction of the total number of organisms potentially present on the carcass or on the surface of the equipment, and do not reflect the real level of hygiene of enterprises (*Blevins et al.,* 2018). Another serious problem is the presence of pathogenic microorganisms (*L. monocytogenes* and *Salmonella*). Work surfaces can be a source for the spread of antibiotic-resistant strains of *Salmonella (Bertolatti et al.,* 2003).

The environmental samples also contained *Brochothrix*, which can grow in an environment with a low oxygen content and a high concentration of carbon dioxide. This means that if *Brochothrix* bacteria get into a meat product packed in a vacuum or a modified gas environment, they can cause its spoilage.

The study of the microbiome of food enterprises brings not only practical benefits to a particular meat processing complex, but also helps to form global databases of taxonomic profiles of microbial communities depending on the geographical location.

5. Conclusion

Sequencing of food microbiomes reveals key characteristics of food safety and quality. Pathogenic bacteria and spoilage microorganisms have been identified, which suggests that the objects and surfaces in the production environment of food enterprises can play a key role in the transfer of microorganisms to food products. The data obtained demonstrate diverse and highly variable communities of microorganisms living on various facilities in the enterprise, which is informative in the context of food safety and spoilage.

References

Bertolatti, D., O'Brien, F. & Grubb, W. (2003). Characterization of drug-resistant *Staphylococcus aureus* isolated from poultry processing plants in Western Australia. *International Journal of Environmental Health Research*, 13, 1, 43–54, https://doi.org/10.1080/0960312021000063322

Blevins, R. E., Kim, S. A., Park, S. H., Rivera, R. & Ricke, S. C. (2018). Historical, current, and future prospects for food safety in poultry product processing systems. Food and feed safety systems and analysis. *Academic Press*, 323–345, https://doi.org/10.1016/B978-0-12-811835-1.00018-X

Bokulich, N. A., Bergsveinson, J., Ziola, B. & Mills, D. A. (2015). Mapping microbial ecosystems and spoilage-gene flow in breweries highlights patterns of contamination and resistance. *Elife*, 4, e04634, https://doi.org/10.7554/eLife.04634

Bulygina, E. S., Kuznetsov, B. B., Marusina, A. I., Kravchenko, I. K., Bykova, S. A., Kolganova, T. V. & Galchenko, V. F. (2002). Study of nucleotide sequences of nifH genes in representatives of methanotrophic bacteria. *Microbiology*, 71(4), 500–508.

Chen, S. H., Fegan, N., Kocharunchitt, Ch., Bowman, J. P. & Duffy, L. L. (2020). Impact of poultry processing operating parameters on bacterial transmission and persistence on chicken carcasses and their shelf life. *Applied and Environmental Microbiology*, 86(12), e00594–20, https://doi.org/10.1128/AEM.00594-20

Cobo-Díaz, J. F., Alvarez-Molina, A., Alexa, E. A., Walsh, C. J., Mencía-Ares, O., Puente-Gómez, P., Likotrafiti, E., Fernández-Gómez, P., Prieto, B., Crispie, F., Ruiz, L., González-Raurich, M., López, M., Prieto, M., Cotter P. & Alvarez-Ordóñez A. (2021). Microbial colonization and resistome dynamics in food processing environments of a newly opened pork cutting industry during 1.5 years of activity. *Microbiome*, 9, 204, https://doi.org/10.1186/s40168-021-01131-9

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26, 2460–2461, https://doi.org/10.1093/bioinformatics/btq461

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) (2016). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA Journal*, 14(12), 4634, 231.

Ercolini, D. (2013). High-throughput sequencing and metagenomics: moving forward in the culture-independent analysis of food microbial ecology. *Applied Environmental Microbiology*, 79, 3148–3155, https://doi.org/10.1128/AEM.00256-13

Frey, B., Rime, T., Phillips, M., Stierli, B., Hajdas, I., Widmer, F. & Hartmann, M. (2016). Microbial diversity in European alpine permafrost and active layers. *FEMS Microbiology Ecology*, 92, 3, https://doi.org/10.1093/femsec/fiw018

Lozupone, C. & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, 71(12), 8228–8235, https://doi.org/10.1128/AEM.71.12.8228-8235.2005

Magoč, T. & Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21), 2957–2963, https://doi.org/10.1093/bioinformatics/btr507

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41(Database issue), D590–D596, https://doi.org/10.1093/nar/gks1219

Ricke, S. C., Dittoe, D. K., Brown, J. A. & Thompson, D. R. (2022). Practical opportunities for microbiome analyses and bioinformatics in poultry processing. *Poultry Science*, 101(5), 101787, https://doi.org/10.1016/j.psj.2022.101787

Rognes, T., Flouri, T., Nichols, B., Quince, C. & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, 4, e2584, https://doi.org/10.7717/peerj.2584

Stellato, G., La Storia, A., De Filippis, F., Borriello, G., Villani, F. & Ercolini, D. (2016). Overlap of spoilage-associated microbiota between meat and the meat processing environment in small-scale and large-scale retail distributions. *Applied and Environmental Microbiology*, 82(13), 4045–4054, https://doi.org/10.1128/AEM.00793-16

Yang, H., Huang, X., Fang, S., Xin, W., Huang, L. & Chen, C. (2016). Uncovering the composition of microbial community structure and metagenomics among three gut locations in pigs with distinct fatness. *Scientific Reports*, 6, 27427, https://doi.org/10.1038/srep27427

Yu, Z., Joossens, M. & Houf, K. (2020). Analyses of the bacterial contamination on Belgian broiler carcasses at retail level. *Frontiers in Microbiology*, 11, 539540, https://doi.org/10.3389/fmicb.2020.539540

Zhang, X., Peng, Z., Li, P., Mao, Y., Shen, R., Tao, R., Diao, X., Liu, L., Zhao, Y. & Luo, X. (2020). Complex internal microstructure of feather follicles on chicken skin promotes the bacterial cross-contamination of carcasses during the slaughtering process. *Frontiers in Microbiology*, 11, 571913, https://doi.org/10.3389/fmicb.2020.571913

Zwirzitz, B., Wetzels, S. U., Dixon, E. D., Stessl, B., Zaiser, A., Rabanser, I., Thalguter, S., Pinior, B., Roch, F. F., Strachan, C., Zanghellini, J., Dzieciol, M., Wagner, M. & Selberherr, E. (2020). The sources and transmission routes of microbial populations throughout a meat processing facility. *NPJ Biofilms and Microbiomes*, 6(1), 26, https://doi.org/10.1038/s41522-020-0136-z