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IDENTIFICATION AND CHARACTERIZATION OF MICROBIAL DIVERSITY ASSESSED BY 16S SEQUENCING ON STAINLESS STEEL, POLYPROPYLENE AND POLYURETHANE SURFACES AFTER PRE-OPERATIONAL CLEANING IN A FISH SLAUGHTERHOUSE

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The contamination of product contact surfaces during industrial processing represents a challenge for industries, as it can result in the transfer of microorganisms to the final products, even after Standard Procedures of Operational Hygiene (SSOP). This transfer not only promotes the spread of pathogenic microorganisms, but also accelerates food spoilage and many species coexist in the same environment and can form multispecies biofilms. Our study aimed to identify and characterize the existing microbial diversity in a tilapia fillet slaughter and processing plant right after SSOP. The samples were collected at five different points over ten consecutive weeks through *swabs* in an area of 400cm² per collection point. The collection points were distributed throughout the cutting room of the fish slaughterhouse, and all of them maintained direct contact with the raw materials. After collection, the *swabs* were stored in *Falcon tubes* containing 0.1% peptone saline solution and polysorbate 80 until the beginning of the analysis. The tubes containing the *swabs* were subjected to vortex orbital agitation and 0.1µl was cultured by the spread plate technique in Petri dishes containing PCA agar, with incubation at 36±1°C/24±2h. From the surface growth, 3mL of sterile saline solution was added to the cultures, which were removed from the agar by scraping and homogenization with the aid of a sterile pipette tip. After this step, the liquid was transferred to 5mL Eppendorf tubes and frozen at -20°C. The alpha and beta diversities were evaluated by the Shannon\'s diversity index, Observed Features, Faith\'s Phylogenetic Diversity and Evenness, Jaccard distance, Bray-Curtis distance, unweighted UniFrac distance, and weighted UniFrac distance for the different materials. Of the 50 samples collected, only 28 showed plaque growth and enough DNA for evaluation by the 16S rRNA gene sequencing technique. The main families we identified were: Staphylococcaceae (18.8%), Moraxellaceae (16.9%), Microbacteriaceae (14.0%), Enterobacteriaceae (12.3%) and Bacillaceae (8.5%) and the main genera were *Staphylococcus* (27.7%), *Acinetobacter* (17.2%), *Bacillus* (12.5%), *Pseudomonas* (9.6%) and *Enterococcus* (7.1%). Through the sampling carried out in this study and the performance of the 16S rRNA gene sequencing technique, it was possible to verify the alpha diversity, bringing relevant information about the environmental microbiota of a

slaughterhouse and tilapia filleting, especially about the permanence of bacteria considered spoilage, such as those of the genus *Pseudomonas* and *Acinetobacter*; and potentially pathogenic bacteria, such as those of the genus *Bacillus*, *Staphylococcus* and *Enterococcus* even after the adopted hygiene protocols. From the results obtained, it was possible to verify that the diversity of microbial genera in clean surfaces was significant and that it is necessary to study the factors related to the persistence of these microorganisms on the evaluated surfaces, suggesting the elaboration and implementation of cleaning protocols in order to reduce surface contamination and the formation of biofilms, fundamental variables to ensure the production of safe and quality food.

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