Profiling of microbial populations as tool for meat quality and safety

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I. INTRODUCTION

Microbiological testing is commonly employed to verify hygienic conditions during slaughtering, as well as downstream operations in meat transformation, and to help in the management of food safety. Commonly, testing is focused on select microbial groups (indicator microorganisms) or target pathogens. International and national guidelines aid producers in setting internal standards regarding load/concentration of indicator/pathogenic microorganisms within the context of food safety management systems.

Microbiological testing has in recent times expanded its scope thanks to the introduction of Next Generation Sequencing (NGS) applications. Amplicon sequencing is employed to get insight into the ecology, in terms of taxonomy, of complex microbial communities, while metagenome sequencing is used when information regarding potential functions of the microbial communities is desired. Such methodologies are applied both for food and environmental samples and are rapidly expanding in various sectors of the food industry, including the meat sector.

This paper provides examples of potential applications of NGS, coupled with classic microbiological testing, in the meat sector.

II. MATERIALS AND METHODS

Meat and environmental samples (from processing facilities) were collected using standard procedures. Samples were transferred in the lab under refrigerated conditions and within 2 hours from collection. Classic microbiological analyses were immediately performed while aliquots for nucleic acid (DNA or RNA) extraction were in parallel collected and appropriately conserved. Nucleic acid extraction was performed using commercial kits and 16S rRNA encoding gene amplicon sequencing (V3-V4 region) was performed. Sequencing data were treated using the QIIME platform and statistical analysis was performed by R. For more detailed information on materials and methods refer to Botta et al. 2020 [1] and Botta et al. 2023 [2].

III. RESULTS AND DISCUSSION

Figure 1 depicts the shift in the microbial communities before and after standard cleaning and sanitation procedures in a commercial beef slaughterhouse facility. As can be seen, the microbial communities residing on surfaces of the slaughterhouse before and after cleaning/sanitation are distinct. In this case, amplicon sequencing provided a global overview of the microbiota and how it is influenced by common activities in the facility.

Figure 2a shows the effect of ozone treatment, applied following standard cleaning and sanitation procedures, on the microbial communities of surfaces in a beef slaughterhouse facility. Ozone treatment (12 hours of gaseous treatment at 20 ppm) profoundly and differentially influenced certain taxa. *Carnobacterium*, *Pseudomonas* and *Brochothrix* significantly decreased while *Staphylococcus aureus* showed an increase although not significant. Importantly, the trends identified through sequencing could be confirmed by viable counts, as shown in Figure 2b. Metaxonomic evaluation clearly indicated what could be the outcome in terms of modification of the residing microbiota when innovative microbiostatic/microbiocidal interventions are applied in a processing plant.

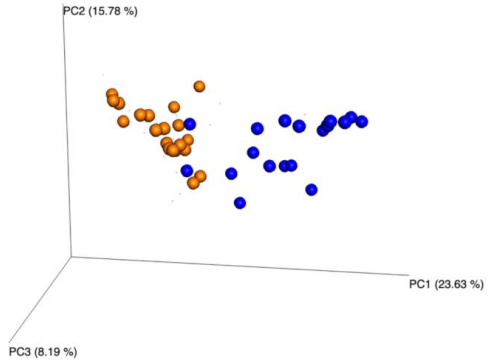

Figure 1. PCoA chart displaying the Weighted UniFrac distance matrix (β-diversity) of a slaughterhouse environment before (blue) and after (orange) cleaning-sanitizing procedures; BC and ACS are different communities (P < 0.001 [FDR adjusted]; ANOSIM and ADONIS tests) [1].

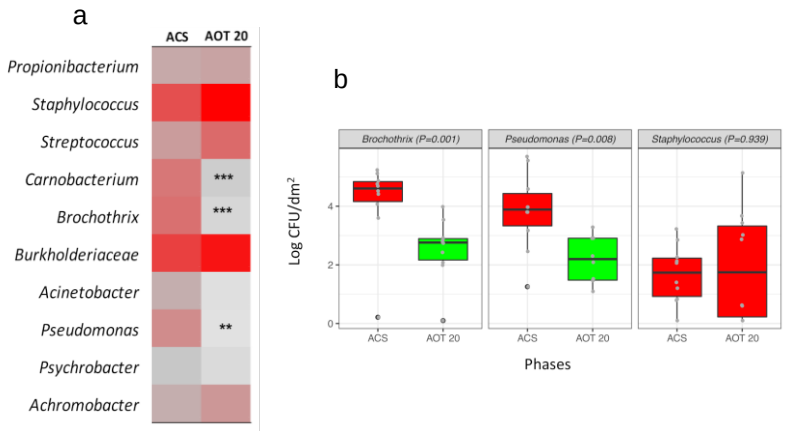

Figure 2. Pseudo-heatmap (a) summarizing the abundance variations of the 10 core OTUs that occurred during the ozone treatments; asterisks highlight significance decrease of relative abundance. Viable counts (**b**) of *Brochothrix*, *Pseudomonas* and *Staphylococcus* before (ACS) and after (AOT 20) a 20 ppm ozonation. Box-plot colors highlight significant differences between ACS and AOT 20 counts. Modified from [1].

When a metataxonomic approach was employed to explore the microbiota of individual carcasses at slaughtering, it was observed that the microbial communities on the carcass surface varied depending on the origin (animal) (Figure 3a). In addition, a comparison was made between the taxa detected on the carcasses before and after cooling. A higher number of unique taxa was observed following a cooling phase (24 hours at 2-4 °C) (Figure 3b) and was mainly represented by psychrotrophs (data not shown).

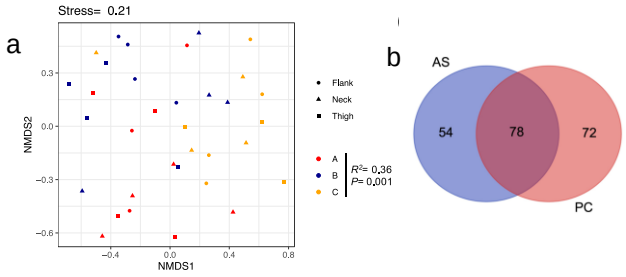

Figure 3. Biplot (**a**) of the Non-Multidimensional Scaling (NMDS) analysis coloured by animal origin, which significantly discriminated the samples. Venn diagrams (**b**) displaying the shared taxa between animals (A, B, C), temporal phases (After slaughtering, AS and post cooling, PC) and sampling areas (Neck, Flank, Thigh). Modified from [2].

IV. CONCLUSION

The examples here presented highlight the depth of information that can be obtained through the application of NGS methodologies in food microbiology. It is now possible to consider food and environmental samples as small ecosystems and through a microbial ecology perspective, study in detail the interactions among microbial groups and how they may be influenced by intrinsic and extrinsic factors that are presented along the production chain.

ACKNOWLEDGEMENTS

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