Impact of the use of lactic/diacetic acids on the microbial diversity of white pudding samples during ageing under various conditions, with the use of next-gen sequencing and targeted metagenomic approach.

Bernard Taminiau[1], Laurent Delhalle[2], Cristina Rodriguez[1], Carine Nezer[2], Sébastien Crèvecoeur[1], Débora Piccolo[2] and Georges Daube[1]

1. Université de Liège, Faculté de Médecine vétérinaire, Département des Sciences des Denrées alimentaires, Liège, Belgique
2. Quality Partner S.A., Reserch & Development Department, Rue Hayeneux, 62 B-4040 Herstal, Belgium

INTRODUCTION

Food products represent great biotopes for bacteria. The optimization of foodstuffs conservation through a better understanding of those biotopes and their spoilage. The current techniques of new generation sequencing give a new dimension to the microbial ecology, through the metagenomic analysis of individuals' large number, within a mixed microbial population.

OBJECTIVES

Our goal is to demonstrate that this technology can be applied to observe effects of food preservatives, usually used to prevent the growth of pathogen, on the overall diversity and evolutions of the bacterial populations during storage of a cooked meat product.
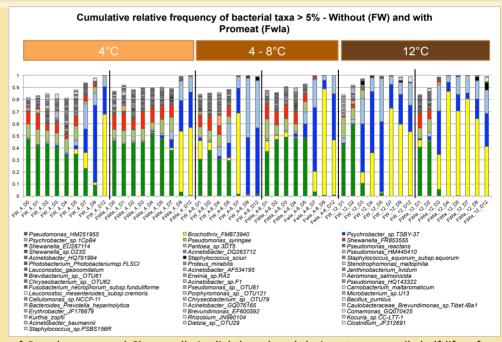
MATERIAL ET METHODS

150 fresh traditional white pudding samples were stored under normal atmosphere in different conditions of temperature (4°C, 12°C and 4°C {2 days} - 8°C {10 days}) until 5 days after shelf-life. Half of the samples were treated with lactic acid / diacetic acid mix (1.8% w/w) (promeat plus, Galactic SA) for 1 minute before storage. Each day, total bacterial DNA was extracted from samples of each conditions and a sequencing library was generated, targeting the V1-V3 region of the 16S rDNA. Libraries were sequenced on a GS junior sequencer using Titanium technology. A Bioinformatic pipeline combining Mothur, Blast and Stamp was used to assign a taxonomical identity to the sequences and to obtain the bacterial population proportions of the samples.

RESULTS

The metagenomic analysis of the samples shows that the bacterial contaminants from the day 0 sample to the post-shelf life sample undergo discrete modifications. As expected, the storage temperature has a strong impact on the bacterial population which undergoes a shift after which spoiling bacteria dominate the matrix.

The targeted metagenomic sequencing approach coupled to careful taxonomical identification enabled us to identify 619 different bacteria taxa belonging mainly to 3 phyla, the actinobacteria, the firmicutes and the proteobacteria. Among the observed taxa, more than 280 do not belong to a known and characterized bacterial species.

Cumulative relative frequency of bacterial taxa > 5% - Without (FW) and with Promeat (Fwla)

For the storage at 4°C, the initial dominance of *Pseudomonas* and *Shewanella* is slightly reduced during storage until shelf life, after which it drops to be replaced by *Brochothrix* and *Psychrobacter*. The main statistical difference observed, following the addition of the preservation treatment, is the delay on the onset of the *Psychrobacter* populations development.

During the ageing assay (2 days at 4°C followed by 10 days at 8°C), the analysis underlines the influence of the temperature change on the onset of the *Brochothrix* and *Psychrobacter* dominance compared to the entire 4°C storage. Again, the preservation treatment delays this onset regarding *Psychrobacter*. Finally, at an abusive 12°C temperature, samples are quickly dominated by the *Psychrobacter/Brochothrix* pair after 2 days of storage. In this case, the lactic acid mix does not appear to be of any effective use.

The power of this method is sufficient to study minor taxa are also statistically influenced by the preservation treatment when we compared samples from the same storage period. This analysis allowed us to observe shift even within bacterial genus. For example, after 7 days at 4°C (shelf life date), *Psychrobacter* sp. TSBY-37 is not only less abundant in the Fwla sample, but it is also the case for other different *Psychrobacter* populations like *Psychrobacter* sp. 1CpB4.

CONCLUSIONS

This work shows that metagenomic analysis can be use to efficiently monitor the effects of additives on the spoilage bacterial populations of food products. This method delivers robust identification of the microflora but also DNA sequences of non characterized microbes, which can be used to prospect for new possible bacterial species. This tool can help additive producers to better characterize the effects of their products and open new perspectives for the food industry.