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# MODELLING THE DEVELOPMENT OF POTENTIAL SPOILAGE AND BIOPRESERVATIVE MICROORGANISMS ON WHITE PUDDING IN DIFFERENT CONDITIONS OF TEMPERATURE BASED ON CLASSICAL MICROBIOLOGY AND 16S rDNA METAGENOMIC

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## INTRODUCTION

Food spoilage is more and more a matter of concern because of the economical loss and the non-selling of impaired products. The deterioration mainly is due to spoilage microorganisms who grow on the foodstuff and degrade it. The result can be a product with off odours, a disgusting aspect and off flavours. But this situation can easily be avoided by storing it in a temperature below 4°C. The producer must keep in mind that the consumer's fridges are rarely at 4°C or less. This reality has to be taken in account by professionals for the establishment of the Used-By-Date (UBD).

## OBJECTIVES

In this sutdy we propose to observe the growth of potential spoilage or biopreservative bacteria on white pudding with the goal to illustrate the behaviour of theses organisms in a temperature higher than 4°C or during a break of 4h at room temperature.

## MATERIAL

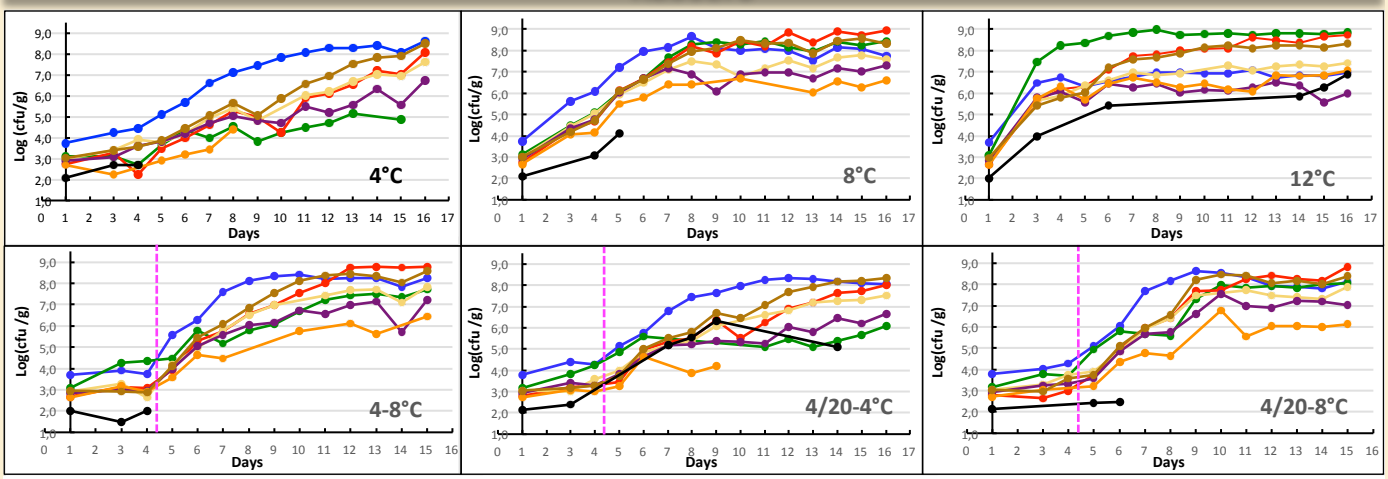
Strains used in this study were isolated in the laboratory from white pudding stored 7 days at 4°C and 14 days at 8°C :

- *Carnobacterium maltaromaticum*
- *Lactobacillus fuchuensis*
- *Lactobacillus graminis*
- *Lactobacillus oligofermentans*
- *Lactococcus lactis*
- *Leuconostoc mesenteroides*
- *Raoultella terrigena*
- *Serratia* sp.


## METHODS

The white puddings were inoculated by soaking them 2 min in a bath of sterile water containing all the strains with the goal of reaching a concentration of 3 ± 1 log of CFU/g on the product. After a drying of 10 min, they were packed under modified atmosphere ($CO_2$ 30% / $N_2$ 70%) and stored at different conditions of temperature : constant 4°C (4°C), constant 8°C (8°C), constant 12°C (12°C), 1/3 4°C – 2/3 8°C (4-8°C), 1/3 4°C – break during 4h at 20°C – 2/3 4°C (4/20-4°C) and 1/3 4°C – break during 4h at 20°C – 2/3 8°C (4/20-8°C). Each day during 16 days, 25 g of product was stomached in 225 mL of physiological water. From this mix, a total count was made on PCA (Plate Count Agar) at 22°C during 48h and an amplicon sequencing analysis based on the V1-V3 fragment of the 16S rDNA was realized on MiSeq apparatus (Illumina) (2x300b).

## RESULTS




A combination was made between the total count of the microflora on PCA at 22°C and the proportions of each strain given by metagenomics, in order to obtain absolute counts for each strain. During the storage at 4°C, the microorganisms didn't clearly achieve the stationary phase in contrast with the 8°C and 12°C storage where they reach it in 7 days and 4 days respectively. *Carnobacterium maltaromaticum* was the dominating strain at 4°C while *Lactococcus lactis* was the one at 12°C, both attaining 8,5 log cfu/g in the end of the storage. The graph at 4°C shows that *Lactococcus lactis* population only reached 5 log cfu/g while the same organism grew until 8 – 9 log cfu/g at 8°C and 12°C. The less developped strains were *Lactobacillus oligofermentans* (under the detection level), *Serratia sp.* and *Raoultella terrigena*. This result is interesting considering the fact that *Serratia sp.* and *Raoultella terrigena* are enterobacteria with a spoilage potential. A hypothesis can be made on a biopresevative effect of the lactic acid bacteria that hinders the development of these microorganisms. An expecting effect was the boost of the strain growth after the transition from 4°C to 8°C that increase their exponential phase and their growth rate. The same effect was observed with the breaks of 4h at 20°C where the growth rate of the strains increased during the days following the rupture. *Lactobacillus oligofermentans* who was not detectable in 4°C and 8°C graphs after 5 days shows a multiplication after the break in the condition 4/20-4°C.

## CONCLUSIONS


The 16S rDNA targeted metagenomics was a powerful tool for the monitoring of a microbial community at species level on a food matrix. It enables us to see the growth of each strain individually and to compare them to one another. The behaviour of the strains at the different temperature conditions clearly shows the importance of keeping the cold chain at 4°C. But even in this low temperature, *Carnobacterium maltaromaticum* achieves a mean of 8 log cfu/g 5 days earlier than other species. Further studies will focus on the confirmation of the spoilage or biopreservative status of the strains by challenge-tests with chemical and sensory analysis.