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The 16S rDNA metagenetic monitoring of refrigerated food products for understanding the kinetics of microbial subpopulations at different storage temperatures: the example of white pudding

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INTRODUCTION

For a clear understanding of the mechanisms that lead to the spoilage of food products, the classical microbiology is not sufficient enough. Fortunately, molecular technologies (like high throughput sequencing methods) can elucidate the microbial communities, including the identification and quantification of culturable and non-culturable organisms, at a much higher resolution than was previously possible with culture-based methods. But spoilage is a complex process resulting mostly of bad temperature storage and bacterial functions that are not all fully understood.

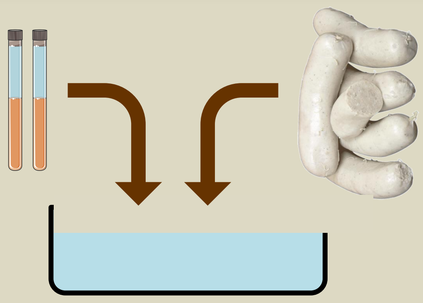
OBJECTIVE

With the aim to avoid food waste and economical loses, it is important for the industrial to have a better understanding of the spoilage mechanism of the microbiota of their products, considering the storage parameters. The present work proposes to follow the evolution of the main microflora's components in white pudding, a typical Belgian meat product. For doing this, a mix of strains was inoculated on the meat product by challenge tests and stored at different temperatures, representative of good or bad practices. The evolution of bacterial flora has been followed each day by combining classical microbiology and 16S rDNA metagenetic analysis. Quantitative PCR (qPCR) analysis targeted on corresponding bacterial genera was used in order to validate the metagenetic approach.

MATERIALS AND METHODS

Inoculated strains :

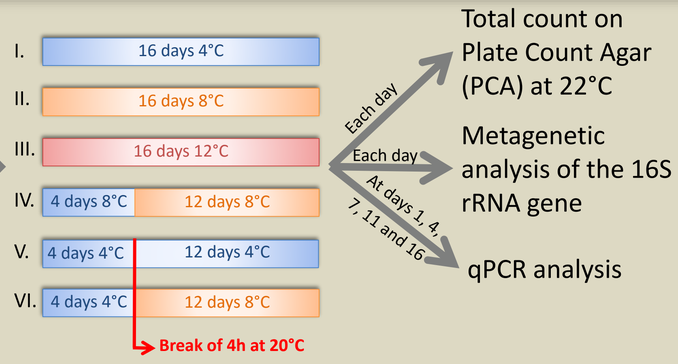
- *Carnobacterium maltaromaticum*
- *Lactobacillus fuchuensis*
- *Lactobacillus graminis*
- *Lactobacillus oligofermentans*
- *Lactococcus lactis*
- *Leuconostoc mesenteroides*
- *Raoultella terrigena*
- *Serratia* sp.



The white-pudding were soaked 2 min in a bath of sterile water containing a mix of the eight strains at the same concentration with the goal of reaching a global concentration of 3 ± 1 log *CFU.g*$^{-1}$ on the product

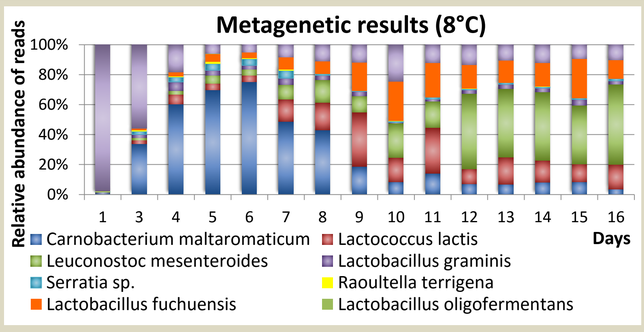
Drying step of 20 min at 10 °C

Two white puddings were packed in a tray (PP/EVOH/PP) under modified atmosphere (*CO2* 30 % / *N2* 70 %)



Samples were stored at different temperature, constant or dynamic : I. 4°C; II. 8°C; III. 12°C; IV. 4-8°C; V. 4/20-4°C; VI. 4/20-8°V

RESULTS



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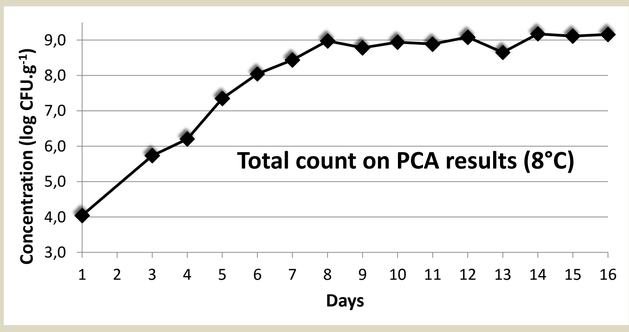

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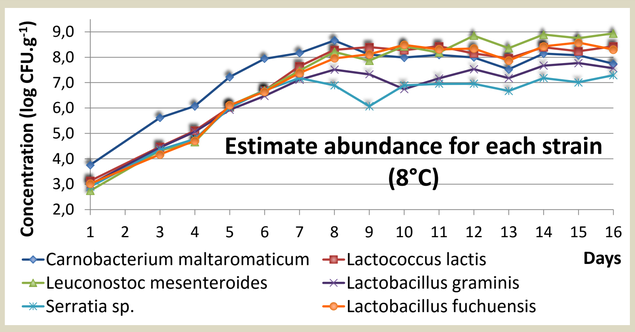

A combination was made between the PCA results of the microflora at 22 °C and the relative proportions of strains given by metagenetic in order to obtain estimate counts for the strains. For this, estimated value of live bacteria was obtained by the percentage of metagenetic results combined with the PCA real values (the 8°C condition above for example). These data were used to obtain growth parameters for each strains and conditions tested but for a better readability, *Raoultella terrigena* and *Lactobacillus oligofermentans* were suppressed because they were often under the detection level for the metagenetic analysis.

Growth parameters obtained for the constant temperature conditions

4 °C	Nmax[a]	Stationary phase[b]	µmax[c]	Class[d]
C. maltaromaticum	8.6	12	0.07	D
Lb. fuchuensis	8.5	16	0.05	S
Lb. graminis	7.6	16	0.03	S
Ln. mesenteroides	8.1	16	0.03	S
Lc. lactis	4.9	12	0.05	I
Serratia sp.	6.7	12	0.04	I

8 °C	Nmax[a]	Stationary phase[b]	µ max[c]	Class[d]
C. maltaromaticum	8.1	8	0.10	D
Lc. lactis	8.4	10	0.09	S
Lb. fuchuensis	8.3	10	0.09	S
Ln. mesenteroides	8.9	10	0.10	S
Lb. graminis	7.6	8	0.08	I
Serratia sp.	6.7	8	0.10	I

Results allowed the bacterial strain subdivision into three classes (d) :

- D (dominant): the highest growth rate (µmax), a maximal concentration (Nmax) between 8 and 9 log *CFU.g*$^{-1}$, and a stationary phase rapidly reached.
- I (inhibited): lesser or equal growth rate than D but an inferior Nmax value and a growth stopped on the same time that the D species.
- S (subdominant): all other bacterial species that continued to growth when the D organisms reached the stationary phase, with a growth rate generally lesser but a high maximal concentration.

12 °C	Nmax[a]	Stationary phase[b]	µmax[c]	Class[d]
Lc. Lactis	8.9	4	0.25	D
Lb. fuchuensis	8.3	11	0.14	S
Ln. mesenteroides	8.7	11	0.10	S
C. maltaromaticum	7.0	4	0.10	I
Lb. graminis	7.4	4	0.11	I
Serratia sp.	6.0	4	0.12	I

a: bacterial concentration at day 16 (Nmax, log CFU.g^{-1}); b: time to reach the stationary phase (days); c: maximal bacterial growth rate (µmax, *h-1*).

Comparaison of growth rates during exponential phase for the dynamic temperature conditions

	4°C VS. 4-8°C	4°C VS. 4/20-4°C	4-8°C VS. 4/20-8°C
C. maltaromaticum	4-8>4**	Ø	Ø
Lc. lactis	4-8>4**	NA	Ø
Lb. fuchuensis	4-8>4**	Ø	Ø
Lb. graminis	4-8>4**	Ø	Ø
Ln. mesenteroides	4-8>4***	4/20-4>4*	Ø
Serratia sp.	4-8>4**	Ø	Ø

Ø: no significant statistical difference; *: significant statistical difference, p-value < 0.05; **: high significant statistical difference, p-value < 0.01; ***: highly significant statistical difference, p-value < 0.001; >: superior value; NA: not valaibale.

An ANCOVA-test was used to compare the growth during the exponential phase of each strain in different dynamic condition of temperature storage.

CONCLUSIONS

In this work, the combination of classical microbiology and molecular tools has allowed to get quantitative results for each strain inoculated to the food product and to study their respective kinetics. The data obtained show different groups inside the ecosystem, interacting the ones with the others, illustrating the Jameson effect (the inhibited vs. the dominant), or not (the subdominant vs. the dominant). Considering the dynamic temperature conditions of storage, the transition from 4°C to 8°C significantly boosted the growth of the strains that reach their stationary phase more rapidly. In contrast, the break of 4h at 20°C did not have a significant effect on the growth rate of the bacterial species during their exponential phase, except for Leuconosotc mesenteroides. These issues show that a no respect of the good storage temperatures is more prejudicial than a break of a few hour at room temperature. Further studies will focus on a deeper understanding of the interaction between the different group of bacterial species highlighted in this work.