

Evaluation of the bacterial diversity and its evolution during storage of fresh beef from British and Belgian origins under different atmosphere and temperature conditions

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Introduction

Food contamination and food spoilage by bacterial organisms have always been a source of concern in food microbiology. Many lactic acid bacteria associated with meat are known for their bactericidal or bacteriostatic activity against other strains, species or genera of bacteria. In this way, the presence of certain lactic acid bacteria in fresh meat could extend the shelf life and improve the microbial stability and safety of this product.

Despite a diverse initial microbial population, vacuum-packaging (VP) or modified atmosphere packaging (MAP), associated with chilling temperatures, will select specific flora in meat. Furthermore, the study of the microflora of chilled beef remains a challenge since some members of the microflora may be missed or not identified by cultivation-based methods.

In this way, the purpose of this study was to evaluate the bacterial diversity and its evolution during storage of fresh beef, depending on its origin, packaging and storage temperature, by using a metagenomic approach.

Materials and methods

Two batches of three vacuum packed beef striploins from United Kingdom and Belgium were obtained from a food wholesaler located in the Walloon Region. Fifteen days after slaughter, the striploins were sliced and individually kept under vacuum: i) at $-1\text{ }^{\circ}\text{C}$ for 30 days; ii) at $+4\text{ }^{\circ}\text{C}$ for 30 days and iii) at $-1\text{ }^{\circ}\text{C}$ for 15 days and then at $+4\text{ }^{\circ}\text{C}$ for 15 days. The bacterial diversity of VP meat was evaluated by a metagenomic approach at 15, 30 and 45 days after slaughter. Furthermore, each 15 days, part of the vacuum packed striploin slices were repacked under modified atmosphere (70 % O_2 /30 % CO_2), stored at $+4\text{ }^{\circ}\text{C}$ for 2 days and at $+8\text{ }^{\circ}\text{C}$ for 5 days in order to simulate storage conditions at retail and home levels, and then analyzed.

Metagenomic analyzes consisted of the following steps: 1) total DNA extraction from 25 g of each sample homogenized in 225 mL of peptone water; 2) DNA pooling (within the same batch); 3) 16S rRNA gene amplification performed by polymerase chain reaction with the universal primers 16S1500F (5'-GAGTTTGATCMTGGCTCAG-3') and 16S1500R (5'-TACGGTTACCTTGTTACGAC-3'); 4) pyrosequencing performed by Quality Partner SA; and 5) data processing.

Results and discussion

Metagenomic analyzes revealed that the origin, atmosphere and temperature conditions influenced the selection of the predominant flora. Vacuum-packaged British samples presented higher concentrations of *Lactobacillus algidus* at the beginning of the experiment than Belgian samples. Furthermore, the development of *Lactobacillus algidus* was favored in British and Belgian samples preserved under vacuum at $-1\text{ }^{\circ}\text{C}$, while a predominance of *Lactococcus piscium* was observed for samples stored at $+4\text{ }^{\circ}\text{C}$ (Figure 1). These microorganisms have already been isolated from beef, but taking into account that the knowledge about these two species is currently limited, it is still not possible to state if the conservability of the tested samples was influenced by the presence of these bacteria.

Moreover, storage under modified atmosphere favored the development of *Leuconostoc gasicomitatum* in both British and Belgian samples (Figure 2). This specie is often associated with spoilage of cold-

stored modified-atmosphere-packaged (MAP) nutrient-rich foods [1, 2]. This result can partially explain the short shelf-life of the samples once they are stored under this condition.

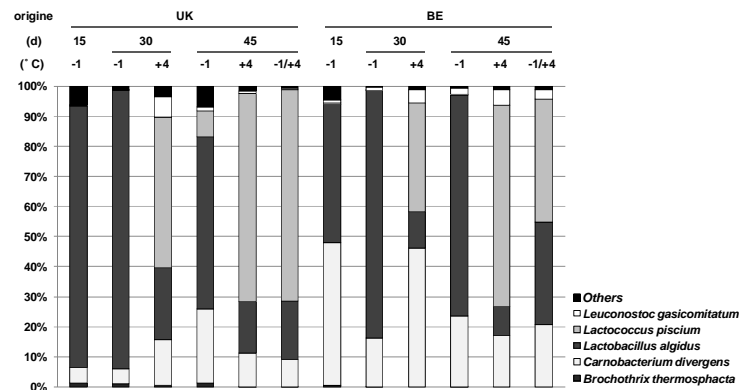


Figure 1. Relative abundance of bacterial genes from samples of British and Belgian fresh beef stored under vacuum at different time x temperature combinations

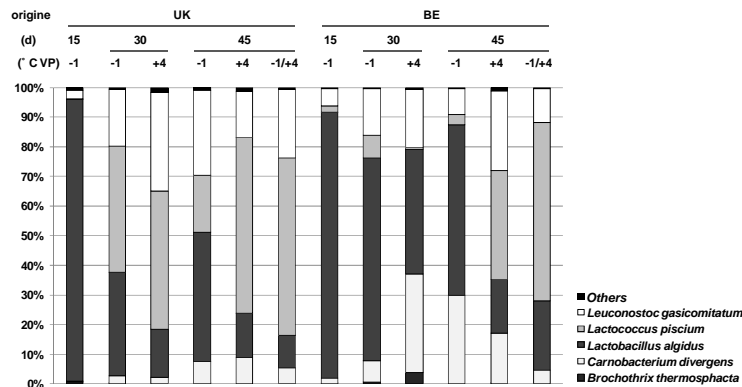


Figure 2. Relative abundance of bacterial genes from samples of British and Belgian fresh beef stored under vacuum, and then under modified atmosphere at different time x temperature combinations

Conclusion

Metagenomics showed to be a useful tool to study the microbial population of a complex matrix since some of the identified genera could not have grown or have grown slowly in culture media commonly used.

In addition, it helped to clarify the evolution of the bacterial ecosystem associated to meat during its storage.

The next step of this study will be to isolate and characterize strains of *Lactobacillus algidus* from meat and to assess their possible bioprotective potential.

Acknowledgments

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References

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