# SPECIES DIVERSITY OF LACTIC ACID BACTERIA FROM CHILLED COOKED MEAT PRODUCTS AT EXPIRATION DATE IN BELGIAN RETAIL

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**Abstract – The bacterial communities of a wide variety of chilled cooked meat products (29 different products), originating from pork and poultry, were subjected to extensive sampling. Samples were stored at 4 °C and analyzed at expiration date. Bacterial isolates were obtained from MRS agar, modified MRS agar, and M17 agar. Next, a procedure consisting of $(GTG)_5$-PCR fingerprinting of genomic DNA followed by numerical clustering was performed and for each cluster the identity of a selection of representative isolates was determined by sequencing of the 16S rRNA gene. Based on the preliminary results, seven lactic acid bacterium (LAB) species were retrieved and belonged to the following genera: *Carnobacterium*, *Leuconostoc*, *Lactobacillus*, and *Vagococcus*. The species *Brochothrix thermosphacta* was often present too. Overall, *Leuc. carnosum* and *C. divergens* were the most common species found on both pork and poultry cooked meats, but pronounced differences between different products were often discovered. Also, some of the samples displayed signs of spoilage at expiration date, indicating potential problems in reaching shelf-life when stored in the Belgian retail chain.**

**Key Words – Lactic Acid Bacteria, meat spoilage, modified atmosphere packaging, cooked meat products.**

## I. INTRODUCTION

Cooked meat products are usually produced from pork, chicken, or turkey and are very popular with consumers. They possess a rather neutral pH, high water activity, and high amount of nutrients, which makes them prone to microbial spoilage, resulting in limited shelf stability [1]. To postpone spoilage, a variety of conservation techniques can be used, including the use of modified atmosphere packaging (MAP) and chilling, as well as the application of spoilage-controlling ingredients (*e.g.*, salt) and additives (*e.g.*, sodium lactate). However, current practices often intend to reduce the amount of salt and additives in view of increasingly stringent consumer demands [1]. As a result of the typical conditions prevailing in the packaged and chilled cooked meat products, specific microbiota develop. Usually, these microbiota mostly consist of psychrophilic and psychrotolerant lactic acid bacteria (LAB), in particular species of the genera *Carnobacterium, Enterococcus, Lactobacillus*, and *Leuconostoc* [2-5]. Some of these LAB have only moderate effects on the sensory status, whereas others have a clear ability to cause spoilage, including slime formation, discoloration, acidification, gas production, and the generation of unpleasant off-flavours. Besides the presence of LAB, *Brochothrix thermosphacta* is regularly found in cooked meat products too, where it can metabolically contribute to spoilage [4]. It remains unclear how and why specific bacterial species develop as a function of the peculiarities prevailing in the product or retail chain, potentially giving rise to differences in the final microbiota and the subsequent spoilage manifestations.

To obtain a better insight into the overall species diversity of the LAB microbiota in cooked meat products, an extensive market survey was carried out on Belgian retail level at expiration date, involving both pork-based and poultry-based products.

## II. MATERIALS AND METHODS

A total of 55 samples (different packages) from cooked meat products originating from 29 different product brands (different logos) were purchased at different supermarkets located in the

agglomeration of Brussels and stored at 4 °C until the expiration dates were reached. Products included pork (34 samples), chicken (19 samples), and turkey meat (2 samples). After measuring of the pH and mechanical treatment (stomaching) of the different samples, the meat-associated microbiota were cultivated using the pour plating method on (i) plate count agar (PCA), (ii) de Man-Rogosa-Sharpe (MRS) agar, (iii) modified MRS (mMRS) agar, and (iv) M17 agar, incubated at 22°C for 5 days. The mMRS medium was equal to standard MRS without acetate and an increased pH of 8.6 [4]. For MRS, mMRS and M17 agar, colonies from the highest dilution were picked up (10-30 %) for further analysis, involving DNA extraction (NucleoSpin®96 Tissue kit; Macherey-Nagel, Duren, Germany) and subjection to $(GTG)_5$-PCR fingerprinting of genomic DNA, as described previously [4]. PCR amplicons were run on agarose gels and the resulting fingerprints were clustered using Bionumerics software (v. 5.10; Applied Maths, Sint-Martens-Latem, Belgium). Representative isolates from the generated clusters were selected and their identity was established by sequencing of the 16S rRNA gene and evaluation with the basic local alignment search tool (BLAST) and NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/).

## III. RESULTS AND DISCUSSION

In general, the pH of the cooked meat products ranged between 5.55 and 6.37. Three out of 55 cooked meat samples showed signs of spoilage at expiration date, including acidity, bloating, and greening. On average, bacterial counts ranged from 6.89 to 8.66 log cfu $g^{-1}$ for PCA, 6.61 to 8.61 log cfu $g^{-1}$ for MRS agar, 6.62 to 8.59 log cfu $g^{-1}$ for mMRS agar, and 6.91 to 8.59 log cfu $g^{-1}$ for M17 agar. The counts indicated that the LAB were the dominating bacteria in cooked meat products.

When analyzing the microbiota originating from MRS, mMRS, and M17 agars, some differences in composition were found (after identification of about one fourth of a total of more than 1000 isolates; results in progress) (Figure 1). Carnobacteria were only slightly better represented on mMRS agar than on MRS agar but did grow particularly well on M17 agar. Whereas mMRS agar is known to allow for better growth of carnobacteria [1], the selectivity of M17 agar for the genus *Carnobacterium* is less described. M17 also allowed for a better recovery of *B. thermosphacta*. Moreover, a minor fraction of lactobacilli was present on all media investigated.

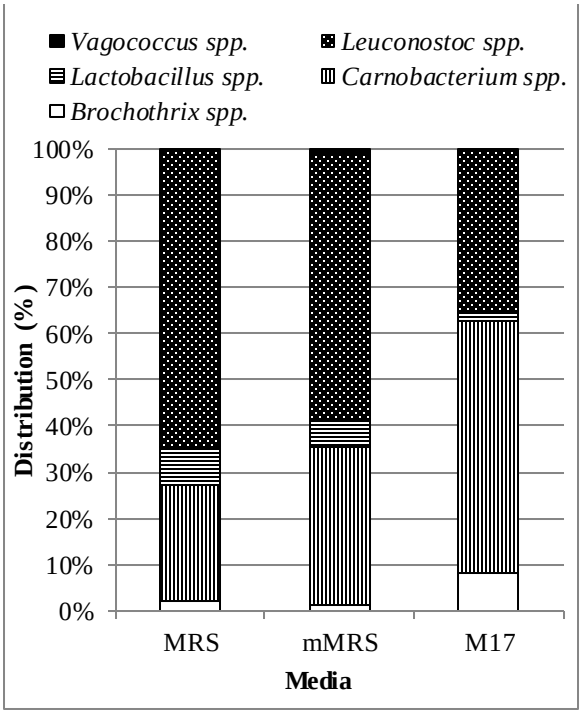


Figure 1. Overall distribution of bacterial genera found on cooked meat products at expiration date in Belgian retail at 4 °C, as isolated from different cultivation media.

On both pork and poultry products, *Leuconostoc carnosum* and *Carnobacterium divergens* were the most frequently recovered LAB species (Table 1). These species are known to have the potential to be dominant in cooked meat products [4-5]. *Leuconostoc gelidum* subsp. *gasicomitatum* was only found on one product brand of oven-baked cooked pork meat. On poultry meat, a few *Lactobacillus sakei* and one *Vagococcus* sp. isolate were found. All the *Lactobacillus sakei* isolates originated from chicken products with added garden herbs or vegetables, whereas a *Vagococcus* sp. was picked up from cooked turkey meat. To the best of our knowledge, this is the first time a *Vagococcus* sp. was found on cooked poultry meat, although it has previously been isolated from non-marinated broiler meat [7]. Other LAB

species found on the cooked meat products that were analyzed till now included *Carnobacterium maltaromaticum* and *Lactobacillus curvatus/ graminis*.

Table 1. Overall distribution of bacterial species found on cooked meat products of pork or poultry origin at expiration date in Belgian retail at 4 °C, as isolated from different agar media.

| Species | MRS | mMRS | M17 |
|---|---|---|---|
| **Pork** | | | |
| *Brochothrix thermosphacta* | 2 | 0 | 4 |
| *Carnobacterium divergens* | 18 | 14 | 10 |
| *Carnobacterium maltaromaticum* | 3 | 5 | 7 |
| *Lactobacillus curvatus/graminis* | 4 | 1 | 0 |
| *Leuconostoc carnosum* | 53 | 33 | 13 |
| *Leuconostoc gelidum* subsp. *gasicomitatum* | 4 | 2 | 1 |
| Total | 84 | 55 | 35 |
| **Poultry** | | | |
| *Brochothrix thermosphacta* | 1 | 1 | 0 |
| *Carnobacterium divergens* | 7 | 9 | 4 |
| *Carnobacterium maltaromaticum* | 6 | 2 | 5 |
| *Lactobacillus curvatus/graminis* | 2 | 3 | 0 |
| *Lactobacillus sakei* | 5 | 1 | 1 |
| *Leuconostoc carnosum* | 31 | 16 | 3 |
| *Vagococcus* sp. | 0 | 1 | 0 |
| Total | 52 | 33 | 13 |

## IV. CONCLUSION

The bacterial communities of chilled and packed cooked meat products in the Belgian retail chain at expiration date consisted mainly of leuconostocs and carnobacteria, with some minor fractions of lactobacilli and *B. thermosphacta*. *Leuconostoc carnosum* was the most dominant species in both pork and poultry products, but some remarkable differences and specificities were found when comparing the different products. This suggests that further analysis of the heterogeneity found may help to understand how certain product characteristics select for particular microbiota.

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