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Variability within the dominant microbiota of sliced cooked poultry products at expiration date in the Belgian retail

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ABSTRACT

Sliced cooked poultry products are susceptible to bacterial spoilage, notwithstanding their storage under modified-atmosphere packaging (MAP) in the cold chain. Although the prevailing bacterial communities are known to be mostly consisting of lactic acid bacteria (LAB), more information is needed about the potential variation in species diversity within national markets. In the present study, a total of 42 different samples of sliced cooked poultry products were collected in the Belgian retail and their bacterial communities were analysed at expiration date. A total of 629 isolates from four different culture media, including plate count agar for the total microbiota and de Man-Rogosa-Sharpe (MRS), modified MRS, and M17 agar as three selective agar media for LAB, were subjected to $(GTG)_5$-PCR fingerprinting and identification by gene sequencing. Overall, *Carnobacterium*, *Lactobacillus,* and *Leuconostoc* were the dominant genera. Within each genus, the most encountered isolates were *Carnobacterium divergens*, *Lactobacillus sakei*, and *Leuconostoc carnosum*. When comparing samples from chicken origin with samples from turkey-derived products, a higher dominance of *Carnobacteria* spp. was found in the latter group. Also, an association between the dominance of lactobacilli and the presence of added plant material and lactate salts was found.

1. Introduction

Cooked poultry products are vulnerable to bacterial spoilage, even under modified-atmosphere packaging (MAP) and when stored in the cold chain (Samelis et al., 2000; Rodríguez et al., 2014; Pothakos et al., 2015). Although less thoroughly characterized than cooked pork products (*e.g.*, Vasilopoulos et al., 2015; Geeraerts et al., 2017), the main bacterial species on cooked poultry products have been agreed upon by several authors (Audenaert et al., 2010; Rodríguez-Pérez et al., 2003; Samelis et al., 2000). In general, lactic acid bacteria (LAB) are predominant, encompassing species belonging to the genera *Carnobacterium*, *Lactobacillus, Leuconostoc* and *Weissella*, besides the occasional retrieval of *Brochothrix thermosphacta.* On raw poultry meat, thus prior to cooking, the same bacteria are present, besides several LAB species belonging to the genera *Enterococcus*, *Lactococcus* and *Vagococcus* (Björkroth et al., 2005; Nieminen et al., 2012b; Rahkila et al., 2012; Pothakos et al., 2015), as well as non-LAB microbiota encompassing *Aeromonadaceae,* enterobacteria and *Pseudomonadaceae* (Herbert et al., 2015; Rouger et al., 2017; Wang et al., 2017). However, despite these previous characterizations, little is known about the variability in relative LAB species representation on cooked poultry products, especially within markets and between types of products.

In the Belgian retail, cooked poultry products are diversified. Although usually prepared from chicken, turkey-derived products are also quite common. Yet, the latter product type has hardly been investigated (Samelis et al., 2000). In addition, several ingredients can be added to these poultry products to generate variability and meet consumer demands. For instance, different kinds of herbs and vegetable particles are often incorporated, with a likely but underexplored impact on the bacterial community structures and associated spoilage manifestation (Säde et al., 2016). Preservatives, such as lactate and acetate salts, may also be applied in cooked meat products (Gonçalves et al., 2005; Benson et al., 2014; Ahmed et al., 2015; Ilhak et al., 2017), as to prolong shelf-life in addition to the commonly imposed hurdles of cooling under MAP (Vasilopoulos et al., 2015).

In the present study, a variety of sliced cooked poultry products that are representative for the Belgian market was analysed

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microbiologically using culture-dependent methods. This variety encompassed chicken *versus* turkey products as well as the presence or absence of plant materials or additives. The larger objective was to acquire knowledge that will contribute to a better understanding of cooked meat as a microbial ecosystem and may aid in the development of novel (bio)protective strategies based on insights into the competitive LAB microbiota.

2. Materials and methods

2.1. Sample acquirement

Acquisition of 42 samples of cooked poultry products was done between October 2014 and June 2015 (Table 1 and Table S1). All samples were marketed as sliced products stored under MAP (unspecified gas mixture and packaging material). They were purchased at three different supermarkets located in the agglomeration of Brussels (Belgium). After acquisition, samples were stored at 4 °C until their expiration dates. Samples from different production batches but carrying the same commercial label, thus also being characterized by identical compositions of ingredients and additives (*i.e.*, E-number compounds) and coming from the same producer, were grouped as 'product type'. Overall, the fifteen product types originated from three production facilities and comprised the following three varieties: (i) 'chicken, natural' (CN; 9 product types, with a total of 25 samples), (ii) 'chicken, with added plant material', containing particles of added tomato, broccoli, garden vegetables, or garden herbs (CP; 4 product types, with a total of 12 samples), and the less common (iii) 'turkey, natural' (TN; 2 product types, with a total of 5 samples). Additives were generally present (Table S1), including sodium nitrite (E250; 42 samples), sodium ascorbate (E301; 42 samples), carrageenan (E407; 37 samples), sodium lactate (E325; 27 samples), sodium citrate (E331; 20 samples), potassium lactate (E326; 19 samples), pyrophosphate (E450; 9 samples), potassium chloride (E508; 6 samples), sodium erythrobate (E316; 3 samples), sodium phosphate (E339; 2 samples), and sodium triphosphate (E451; 1 sample). Relevant label information is represented in Table S1 for all samples.

2.2. General analysis of the samples

At expiration date, a sensorial check of the samples (appearance, smell, and taste) was done for spoilage manifestation. The pH was measured using an InoLab pH7110 m (WTW, Meilheim, Germany) in combination with an insertion pH probe (VWR International, Darmstadt, Germany). For triplicate analysis of the bacterial communities, 10–15 g of sample was decimally diluted with peptone physiological solution [0.85% (m/v) NaCl and 0.1% (m/v) peptone in ultrapure water] and mechanically treated at medium speed for 90 s using a Stomacher 400 (Seward, Worthington, UK). Dilutions were subjected to pour plating on four agar media. Plate count agar (PCA; Oxoid, Basingstoke, UK) was used for analysis of the total overall bacterial diversity. Presumable LAB were determined using de Man-Rogosa-Sharpe (MRS, Oxoid) agar medium that was adjusted to pH 5.9. Modified MRS (mMRS) agar medium was used for the enumeration of carnobacteria, as previously outlined (Geeraerts et al., 2017). In brief, the latter agar medium was made from single ingredients based on the composition of MRS medium, but without the addition of acetate and by increasing the pH to 8.6. For the presumptive presence of lactococci, M17 agar medium (Oxoid) was used. An overlay with soft agar [1.2% (m/m)] was added to all agar media, followed by incubation at 22 °C for 5 d.

2.3. Isolation and identification of bacterial species

Up to 30 isolates were picked randomly from the appropriate dilution for each agar medium (corresponding with 5–30% of the colonies available), transferred into brain heart infusion (BHI) medium (Oxoid), and cultivated at 22 °C for 24–48 h. Cell cultures were stored in a glycerol solution [25% (v/v)] at −80 °C. Prior to genomic DNA extraction, centrifugation was done at 13,793 × *g* for 5 min (Biofuge 13; Heraeus, Osterode, Germany) to collect cell pellets, followed by a washing step with Tris-EDTA-sucrose (TES) buffer (0.2 M sucrose, 0.001 M EDTA, and Tris-base; pH 8.0). Washed cells were lysed with 200 μl of cell lysis solution, consisting of Tris-EDTA-Triton-X-100 (TET) buffer (0.02 M Tris-HCl, 0.002 M EDTA, and 1% Triton X-100; pH 8.0) with 4 mg of lysozyme (Merck, Darmstadt, Germany) and 100 U of mutanolysin (Sigma-Aldrich, St. Louis, MO, USA), and the mixture was incubated at 37 °C for 60 min. This was followed by a secondary treatment with 25 μl of proteinase K solution (29 mg/ml; Macherey-Nagel, Düren, Germany) at 56 °C for 60 min. Finalisation of genomic DNA extraction and purification was done using the NucleoSpin®96 Tissue kit (Macherey-Nagel) as instructed by the manufacturer. Prior to the PCR assays, DNA concentrations were measured with a Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, MA, USA). Next, $(GTG)_5$-PCR fingerprinting was performed using a denaturation step at 95 °C for 5 min, followed by 30 cycles of 1-min denaturation at 94 °C, hybridisation at 40 °C for 1 min, extension at 65 °C for 8 min, and a final step at 65 °C of 10 min (Vasilopoulos et al., 2008). PCR amplicons were run on agarose gels (constant voltage of 60 V during 960 min) and stained with ethidium bromide for 20 min. Isolates were classified and identified using numerical cluster analysis of the $(GTG)_5$-PCR fingerprints of genomic DNA using Bionumerics software (v5.10; Applied Maths, Sint-Martens-Latem, Belgium). Isolates that were representative for the different $(GTG)_5$-PCR fingerprint clusters were selected and identified by primary sequencing of the 16 S rRNA gene from their genomic DNA. Secondary sequencing was done for a narrowed selection of those isolates using the *pheS* gene (De Bruyne et al., 2007; Snauwaert et al., 2013). The resulting gene sequences were processed with the BioEdit Sequence Alignment Editor (v. 7.2.5.0; Ibis Biosciences, Carlsbad, CA, USA) and identified using the basic local alignment search tool (BLAST) and National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/BLAST).

2.4. Statistical analysis

Data were processed in Microsoft Excel and further analysed with IBM SPSS Statistics (v. 20; IBM Corporation, Armonk, NY, USA). A principal component analysis (PCA) was performed using the pH values (divided along eleven classes ranging from 5.3 to 6.4 with a 0.1 interval), the bacterial counts from the four agar media [divided along eight classes ranging from 5.5 to 9.5 log (cfu g^{-1}), with a 0.5 interval], the prevalence of the different bacterial genera, the addition of plant fragments, the use of chicken *versus* turkey, and the number of chemical additives [divided along three classes containing (i) neither E325 or E326, (ii) E325 but no E326, or, (iii) E325 and E326)].

3. Results

3.1. General analysis of sliced cooked poultry samples

No obvious signs of spoilage were found among the different sliced cooked poultry samples at expiration date. The pH values ranged between 5.3 and 6.4, with an average value of 6.1 (Table 1). On seven samples (CN-F1-P1B, CN-F2-P8A, CN-F2-P8B, CP-F1-P1B,