Introduction

The new generation sequencing techniques have given a new dimension to the microbial ecology, through the metagenomic analysis of individuals’ large number, within a mixed microbial population. This methodology can be successfully applied to the field of food microbiology. As an example, we present here a description of the microbial flora content and evolution during the storage of a crude meat and a cooked meat preparation.

Methods

- Samples of pork minced meat and white pudding were stored under various conditions of storage temperature and packaging (see Table 1).
- They were analyzed by classic microbiology (ISO protocols) and total bacterial DNA were extracted at day zero and at the shelf-life limit (given by the meat retailer).
- DNA samples were submitted to rDNA 16S amplicon sequencing (GS-junior Roche) targeting the V5-V6 hypervariable region.
- The bacterial taxonomic identifications were derived from a bioinformatic analysis using the MOTHUR package.

The microbiological analysis (Table 1) show an usual increase in bacterial contamination during storage which is proportional to the storage temperature. This is also observed with the metagenomic analysis.

About 130,000 sequences were obtained from the samples and analyzed for taxonomic identification. Targeting the VS-V6 rDNA region, more than 80% of sequences could be identified to the genus level. The Figure 1 and Figure 2 present respectively the comparison of the proportion of relative bacterial counts in the minced meat and white pudding samples. These counts are expressed as a percentage of the relative bacterial count of the major OTUs (with a size of at least 0.2% of the total population) present in the sample. For each OTU, the taxonomic identity given is the last level of taxonomy shared by at least 80% of the sequences within this OTU.

Results and discussion

Figure 1. Mincing meat sample stored under modified (A) or normal atmosphere (B) packaging

Figure 2. White pudding samples

Several interesting observations can be made on the impact of the storage:
- Storage temperature shows a significant impact on the minced meat samples. The domination of Pseudomonas (FW) and Leuconostoc (MAP) at lower storage temperatures is diminished when abused storage conditions are used (12 °C), as a result of the rise of Brochothrix spp.
- The packaging influence is also revealed by the metagenomic data. In the minced meat samples, Leuconostoc represents the leading contaminant in MAP whereas Pseudomonas was the most prevalent population in the FW samples.
- For the white pudding, MAP50 was compared with the traditional MAP30 composition. At the shelf life limit, Leuconostoc and uncultured Brochothrix invaded both samples. The main difference between the MAP50 and MAP30 samples was a diminution of Listeriaeae in MAP50, compensated by an increase in the presence of Leuconostoc.

The 16S targeted metagenomic analysis employed here gives the same results as the classic microbiological analysis but with much more information regarding the composition of the bacterial flora. Moreover, it sheds light on the diversity of samples containing relatively few bacteria like day zero samples, thus being invaluable for accurately identifying sub-populations and for addressing their potential influence on the spoilage of the meat.