Predictive microbiology combined with metagenomic analysis targeted on the 16S ribosomal DNA : A new approach for food quality

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OBJECTIVES

The food spoilage process is mainly caused by alteration micro-organisms and classical culture-based methods have therefore been used to assess the microbiological quality of food. These techniques are simple to implement but may not be relevant to understand the modifications of the microbial ecology which occur in the food product in response to different changes in the environmental conditions. Metagenomic analysis targeted on 16S ribosomal DNA can bring about a solution to this new need and elucidate microbial community structures, including the identification and quantification of culturable and non-culturable organisms, at a much higher resolution than was previously possible with culture-based methods to provide a picture of the microbial community. Combined with predictive microbiological models, a new approach was investigated to take into account the dynamics of the evolutions of the microbial community in food products. This work describes the application of a metagenomic analysis and predictive microbiology in order to study bacterial populations dynamics in perishable foods under different environmental conditions.

METHODS

White pudding samples, a typical Belgian pork meat product, were packed under food wrap (atmospheric air condition). Durability studies were conducted at 4°C, 12°C and a dynamic temperature profile according to the NF V01-003 standards (4°C (1/3 of the shelf life) - 8°C (2/3 of the shelf life)) during 15 days. The effect of organic acids was also investigated using a lactic acid / diacetic acid mix (1.8% w/w) treatment. At each day of the trials, classical microbiological (total flora, lactic acid bacteria) and 16S rDNA metagenomic analysis were carried out on all these samples. For the metagenomic analysis, a sequencing library was generated, targeting the V1-V3 region of the 16S rDNA. Libraries were sequenced on a GS junior sequencer using Titanium technology. The Bio-informatic pipeline using Mothur, Blast and Stamp was used to assign a taxonomical identity to the sequences and to obtain the bacterial population proportions of the samples (Schloss, Westcott et al. 2009). The major bacterial populations were thus identified and predictive microbiology models (Baranyi and Roberts 1994; Augustin, Zuliani et al. 2005) were used to assess the growth parameters. The model was validated using the data obtained at a dynamic temperature profile.

RESULTS

The metagenomic analysis of the samples shows that the bacterial populations from the day 0 sample to the post-shelf life sample have important modifications. *Brochothrix* and *Psychrobacter* were identified as the dominant flora. As expected, the storage temperature had a strong impact on the

bacterial evolutions. Moreover, the use of lactic acid/diacetic acid reveals the sensitivity of the different populations to the treatment. For the storage at 4°C, the initial dominance of *Pseudomonas* and *Shewanella* is slightly reduced during storage until shelf life, after which it drops to be replaced by *Brochothrix* and *Psychrobacter*. The addition of the preservation treatment has a statistical negative impact on the *Psychrobacter* and *Acinetobacter* populations. During the ageing assay (2 days at 4°C followed by 10 days at 8°C), the analysis underlines the influence of the temperature change on the onset of the *Brochothrix* and *Psychrobacter* dominance compared to the entire 4°C storage. Again, the preservation treatment delays this onset. Finally, at an abusive 12°C temperature, samples are quickly dominated by the *Psychrobacter/Brochothrix* pair after 2 days of storage. In this case, the lactic acid mix does not appear to be of any effective use. Adjustment of primary model was made on the major bacterial populations and simulation was made based on estimated growth rate. The simulations of the three major populations seem to be sufficient for this food product to predict 80 -90 % of the bacterial population at the end of the shelf life in function of the environmental conditions.

CONCLUSIONS AND IMPACT OF THE STUDY

Compared to culture based methods on selective media and previous independent culture techniques, metagenomic analysis combined with predictive microbiology gives more valuable information, and its use could be considered as a technique for quality control or for accurately determining shelf life.

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