Molecular identification of the bacterial populations of steak tartare, a raw consumed meat preparation: a practical use of targeted metagenomic analysis

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Introduction

Steak tartare is a meat dish made with spicy raw beef meat. Highly popular in Belgium and other European countries, it is often consumed with french fries or as sandwich spread. The use of raw meat and the inner composition of this preparation rise its vulnerability to bacterial alteration. A better understanding of the natural bacterial flora present in this meat product will be insightful to master alteration hazards and help steak tartare producers.

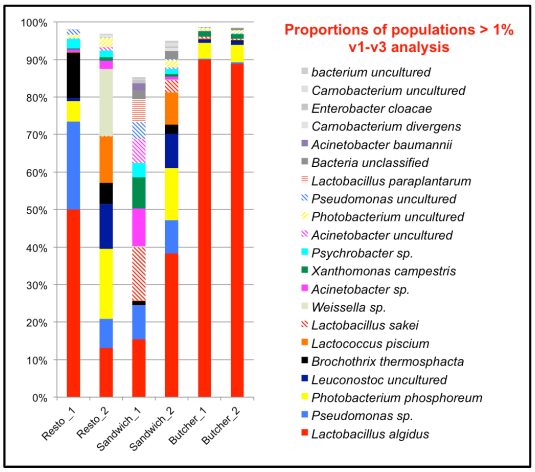
Analysis Scheme

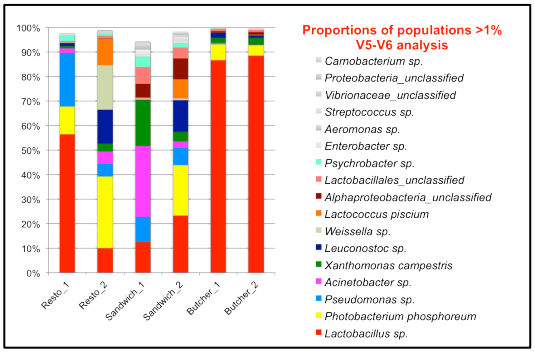
- 6 samples of steak tartare have been harvested: 2 samples from butcher shops, 2 samples from restaurants and 2 samples from sandwich vendors.
- The day of purchase, samples have been analyzed with standardized microbiological analysis (Lactic acid flora, total mesophilic flora and *Pseudomonas* spp.).
- The 6 samples have been submitted to 2 metagenomic analysis targeting the V1-V3 and V5-V5 hyper-variable regions of the 16S rDNA (with the Roche GS-junior sequencing machine).
- The MOTHUR package and BLAST algorithm have been used to attribute a bacterial identification to the sequences.

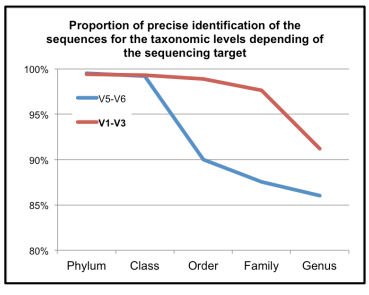
Objectives

- To Identify the bacterial populations found in steak tartare with a targeted metagenomic approach in order to assess its relevance in food microbiology.
- To compare the efficacy of two target sequences for the precise bacterial taxonomic identification : V1-V3 et V5-V6.

Results







The charts to the left show the cumulative proportions of the bacterial populations found in the 6 samples. The taxonomic identity is given by default as "genus species" or "genus sp." when the species is undetermined. If the identification cannot be attributed to the genus level, then the last taxonomic level is given followed by "unclassified". the analysis of the bacteria identified in the samples underline a remarkable biodiversity which appears to be related to the origin of the sample.

- Samples from the butcher shops present a significant contamination level (10^5-10^6 $CFUg^{-1}$) but with a major presence of *Lactobacillus algidus* followed by a lesser contamination by *Photobacterium phosphoreum*.
- Samples from sandwich vendors appear less contaminated (total aerobic mesophilic plate count 10^4 $CFUg^{-1}$) though they possess a higher bacterial diversity (even between both samples). It is likely that this species richness comes from a more complex food matrix (with vegetable contaminants like *Xanthomonas campestris* ou *Acinetobacter* sp.) or from a lesser quality of the samples (presence of known spoiling bacteria like *Leuconostoc* sp. et *Pseudomonas* sp.)..
- Among the 2 samples from restaurants, the sample "resto_2" showed organoleptic alterations(ropy aspect). Both samples were highly contaminated (>10^6 $CFUg^{-1}$) but with different bacterial populations. "Resto_1" differs from "resto_2" with a contamination profile close to the samples from butcher shops, apart from the presence of *Pseudomonas* sp. et de *Brochothrix thermosphacta*. "resto_2" shows a higher bacterial diversity, in particular with spoiling bacteria which are in sufficient numbers to explain the observed alteration.

The comparison of the two targeted sequencing regions reveals that V1-V3 region of the 16S can be used to give a precise taxonomic identification to the genus for more than 90% of the sequences, against 85% for V5-V6 hyper-variable region. Moreover, the mean length of the sequence reads is enough to complete the taxonomic identification with the use of BLAST algorithm to the species level for 83% of the sequences.

Conclusion

- The targeted metagenomic analysis can be used to assess the bacterial biodiversity in steak tartare samples with a taxonomic identification to the genus level for more than 90% of the sequences.
- There are remarkable differences in the microflora, depending on the origin of the matrix. The highest microbiological quality is found in the butcher shops.
- Between the two hyper-variable regions targeted for sequencing, V1-V3 seems to be the best choice in terms of bacterial identification.