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Study of the microbial flora of steak tartare by metagenomic approach

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INTRODUCTION

Steak tartare is a meat preparation prepared with bovine meat after grinding. It is eaten raw, hence representing a potential hazard for the consumer. Surveillance of spoilage during food storage is based primarily on the enumeration of total microbial viable counts. However, microbial analysis alone might not be sufficient for understanding the modifications of the microbial ecology.
Molecular technologies can elucidate microbial community structures. Among the culture-independent techniques, the metagenomic analysis targeting 16S ribosomal DNA has emerged as a powerful tool for studying bacterial composition of various ecosystems.

OBJECTIVES

- **The first objective was to find out if there were differences between steaks tartare in relation with sampling location.**
- **Secondly, we wanted to know which microbial floras were able to grow during the 2-days storage.**

MATERIALS AND METHODS

Sampling

Fifty-eight (58) samples of steak tartare (ST) were collected.

storage 1day at 4°C + 1 day at 8°C

Sample location	Day 0	Day 2
	n	n
Butcheries	7	7
SM1 (pre-packed ST collected in supermarkets)	8	8
SM2 (ST processed in supermarkets)	8	8
Restaurants	6	
Sandwich's shops	6	

Methods

Mesophilic Total Viable counts (TVC) at 30 °C (ISO 4833 method)
Metagenomic analysis targeting the V1-V3 region of the 16S rDNA was performed using the Roche GS junior. Raw sequences were treated by bioinformatics in order to obtain identification and proportion of bacteria in food samples.

Statistics

TVC :
- **non-parametrics statistics (Kruskal-Wallis and Wilcoxon)**

Metagenomics
- **percentage of reads for each OTU**
- **conversion in cfu / g (in relation with TVC results)**
- **Bray-Curtis analysis (dissimilarity)**

RESULTS

TVC results

Sample location	n	Day 0 TVC P50 (min – max)	n	Day 2 TVC P50 (min – max)	W value (significance)
Butcheries	7	7.5×10^4 ($8 \times 10^3 - 2.8 \times 10^7$)	7	3.4×10^5 ($8.8 \times 10^3 - 5.5 \times 10^8$)	23 (NS)
SM1	8	2.95×10^4 ($9.8 \times 10^3 - 3.8 \times 10^4$)	8	3.3×10^4 ($1.4 \times 10^4 - 3.8 \times 10^5$)	9.5 (NS)
SM2	8	3.8×10^4 ($7.6 \times 10^3 - 8.1 \times 10^5$)	8	3.8×10^4 ($1.5 \times 10^4 - 3.6 \times 10^5$)	19.5 (NS)
Restaurants	6	4.05×10^4 ($8.6 \times 10^3 - 1.7 \times 10^5$)		-	
Sandwich's shops	6	1.04×10^5 ($8.2 \times 10^3 - 1.9 \times 10^5$)		-	

Kruskal-Wallis and Wilcoxon : no significant difference was observed

Metagenomics

Metagenomic analysis have identified until 180 bacterial species and 90 genera in some samples depending on the origin.

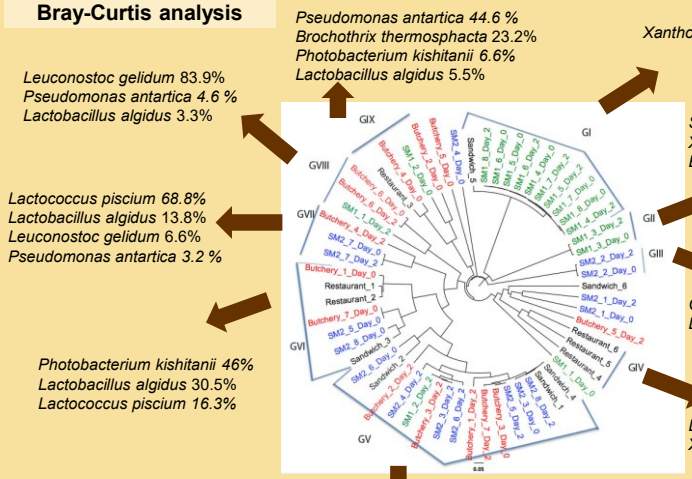
The samples from SM1 were very stable (between D0 and D2).

In other samples, we can observe a modification of the microflora with, in several cases, the development of *Lactobacillus algidus*.

Among all the samples seven major bacterial species are predominant:

***Brochothrix thermosphacta*, *Lactobacillus algidus*, *Lactococcus piscium*, *Leuconostoc gelidum*, *Photobacterium kishitani*, *Pseudomonas* spp., and *Xanthomonas oryzae*.**

Bray-Curtis analysis



Pseudomonas antartica 44.6 %
Brochothrix thermosphacta 23.2%
Photobacterium kishitanii 6.6%
Lactobacillus algidus 5.5%

Xanthomonas oryzae 84.9%

Leuconostoc gelidum 83.9%
Pseudomonas antartica 4.6 %
Lactobacillus algidus 3.3%

Streptococcus thermophilus 44.5%
Xanthomonas oryzae 5.9%
Brochothrix thermosphacta 1.3%

Lactococcus piscium 68.8%
Lactobacillus algidus 13.8%
Leuconostoc gelidum 6.6%
Pseudomonas antartica 3.2 %

Clostridium haemolyticum 84.7%
Lactobacillus algidus 7.1%

Photobacterium kishitanii 46%
Lactobacillus algidus 30.5%
Lactococcus piscium 16.3%

Leuconostoc gelidum 6.8%
Xanthomonas oryzae 5.8%

Lactobacillus algidus 71.2%
Photobacterium kishitanii 14.3 %
Lactococcus piscium 4%
Leuconostoc gelidum 2.1%

DISCUSSION

TVC was comprised between 2.95 x 10^4 cfu/g and 3.4 x 10^5 cfu/g. Seven major bacterial species were present in steak tartare. Nine main groups were observed with dissimalrity analysis.
- ***Xanthomonas oryzae* come mainly from rice, onion, garlic**
- ***Streptococcus thermophilus* from yoghourts or fermented milk product**
- ***Clostridium haemolyticum* (origin: bacteriaemia in cattle ?)**
- ***Brochothrix thermosphacta*, *Leuconostoc gelidum*, *Photobacterium kishitani*, *Pseudomonas* spp: well-known spoiilage organisms of fish and meat**
- ***Lactobacillus algidus, Lactococcus piscium* : controversial role in spoilage of meat or strain related**

CONCLUSIONS

Compared to culture based methods on selective media and previous independent culture techniques, metagenomic analysis combined with the enumeration of psychrotrophic flora gives more valuable information, and its use should be considered as a technique for quality control or for accurately determining the shelf life and the quality of the meat preparations in order to improve their quality.