Study of the microbial flora of freshwater and seawater fish filets in different packaging conditions by metagenomic analysis targeted on the 16S ribosomal DNA
Plan

1. General Context
2. Aim of the study and analyzed samples
3. Results
4. Others samples
5. Conclusions
Fish is spoiling rapidly
⇒ caused by bacterial flora

Pangasius

Haddock
Food microbiological analysis

**Detection methods** (P/A in x g))
- Only for bacterial pathogens
- Always enrichment necessary

**Counting methods** (cfu/g)
- Available for some indicator flora
- Available for some bacterial pathogens
Metagenomics

- 40 Mb data
- 100,000 sequences / run
- 3 days for one run

DNA extractions
Amplification/preparation of libraries
High throughput DNA sequencing
Raw data

Mothur → Blast
Perl script → Normalisation
Data analysis

Food samples

Classical microbiological analysis

Bacterial 16S rRNA gene amplification and barcoded pyrosequencing
Why metagenomics?

**Classical microbiology**

- 1 colony → 1 analysis
- 20 colonies → 20 analysis
- 1 bacterial identification
  ➔ 20 bacterial identifications

**Metagenomics**

- 1 analysis → 10,000 bacterial identifications

Bacterial diversity profile

But are they the ones being sought??
Analyzed samples:

### Seawater: Eglefin
- Day of packaging: Day 0
- End of the shelf life: Day 2
- Plastic wrap: Day 2
- End of the shelf life: Day 6

### Freshwater: Pangasius
- Day of packaging: Day 0
- End of the shelf life: Day 5
- Plastic wrap: Day 5
- End of the shelf life: Day 8

Additional analysis:
- Classical microbiology and metagenomic analysis
The aim of the study

Identification of flora involved in the fish from freshwater and seawater

Day of packaging  End of the shelf life  End of the shelf life

Pangasius  Plastic wrap  MAP

Eglefin

Validation of the shelf life
Results

With classical microbiology:

<table>
<thead>
<tr>
<th></th>
<th>PACC</th>
<th>LAB</th>
<th>PC</th>
<th>SA</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pangasius</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>3.66</td>
<td>&lt;1</td>
<td>&lt;2</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>under FW (5 days)</td>
<td>7.99</td>
<td>3.64</td>
<td>6.85</td>
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<td>3.38</td>
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<tr>
<td>under MAP (8 days)</td>
<td>7.74</td>
<td>6.48</td>
<td>6.32</td>
<td>&lt;1</td>
<td>3.83</td>
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<tr>
<td><strong>Haddock</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>4.3</td>
<td>1</td>
<td>4.04</td>
<td>&lt;1</td>
<td>1.7</td>
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<tr>
<td>under FW (2 days)</td>
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<td>1</td>
<td>5.95</td>
<td>&lt;1</td>
<td>1.9</td>
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<tr>
<td>under MAP (6 days)</td>
<td>5.66</td>
<td>1.3</td>
<td>5.98</td>
<td>&lt;1</td>
<td>3.26</td>
</tr>
</tbody>
</table>

ACC (Psychotropic Aerobic colony counts), LAB (lactic acid bacteria), PC (Pseudomonas counts), SA (Staphylococcus aureus) and EC (Enterobacteriaceae counts). FW: Food Wrap; MAP: Modified Air Packaging.

And that’s it...
Results

With metagenomics:

- 45 different bacteria species identified for the pangasius
- 43 different bacteria species identified for the haddock
- Significant variations of the initial flora at the end of the shelf life depending on the fish and packaging
- Relation between spoilage and the activity of some bacterial species
Pangasius (freshwater)
Haddock (seawater)

Freshness indicator

Spoilage flora

Plastic wrap

MAP
Conclusions

• Few informations with classical microbiology
• Identification and quantification of all bacteria species is now possible with metagenomics
• Evaluation of specific function of micro organisms on food products => expertise

Metagenomic tools could adequately determine the duration of shelf-life
Perspectives

Knowing and controlling the total supply chain who can influence the total shelf life

• Raw material biology (wild catch – farmed)
• Hygiene (PR programs)
• Temperature (PR programs)
• *Carnobacterium* strains could be added on such lightly preserved product
• Environmental factors could be modified (pH, lactic acid, packaging)

-> A NEW APPROACH OF FOOD QUALITY
Others samples

- Food samples
- Sillages
- Intestinal flora from guts of animals
- Sea water
- Seeds
- Virus
- ...

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