

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

Context

Fish is spoiling rapidly

⇒ caused by bacterial flora



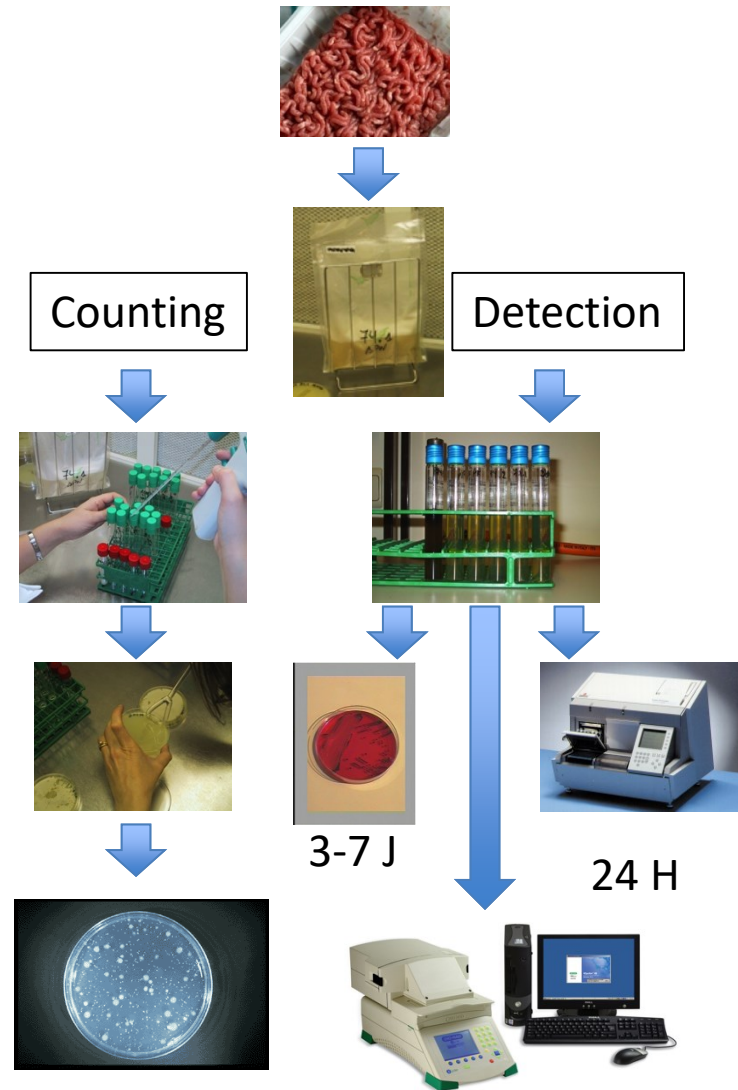
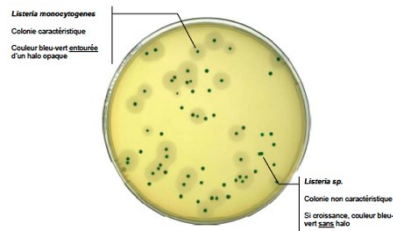
Pangasius



Haddock

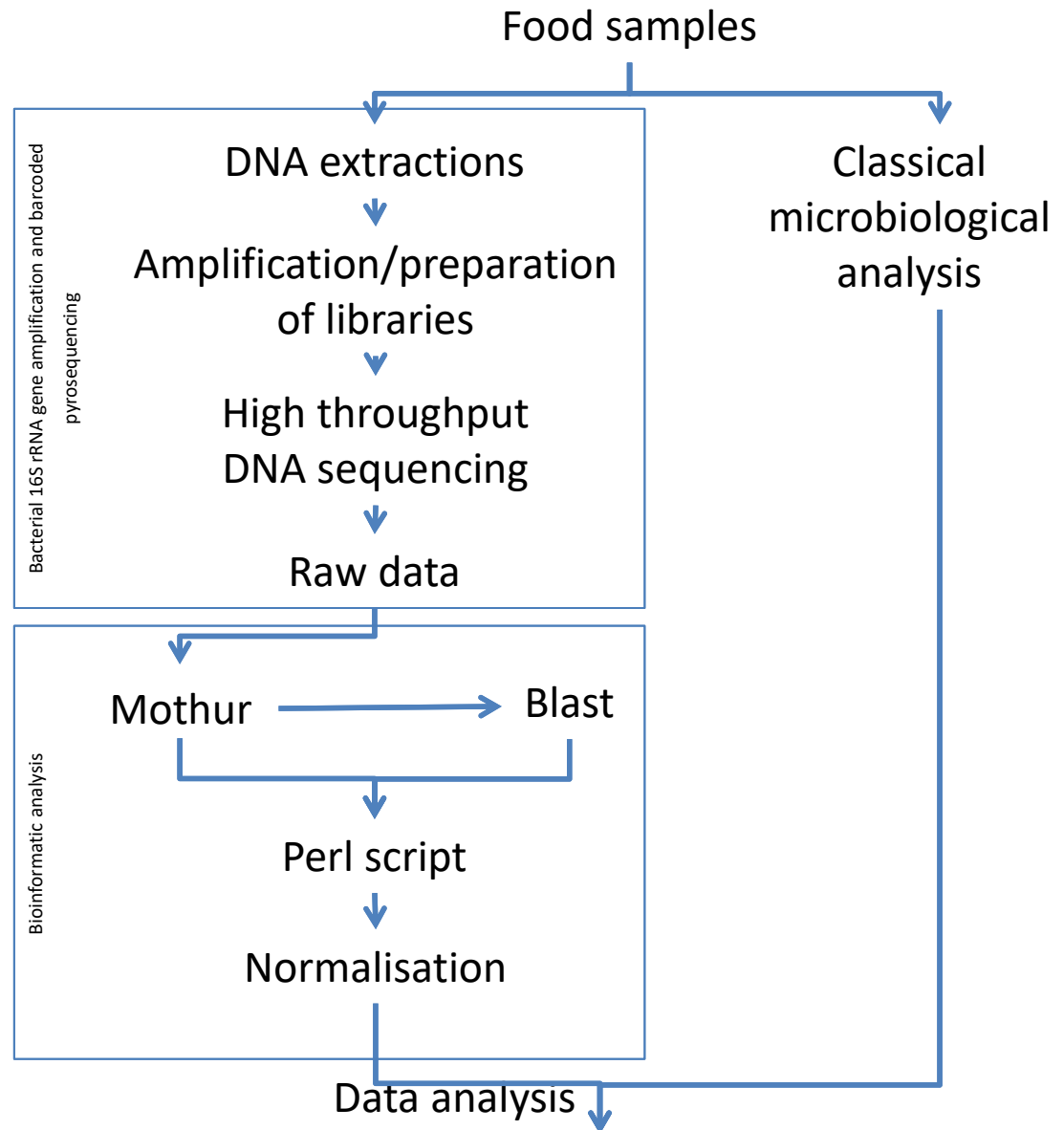
Food microbiological analysis

- **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



Why metagenomics ?

Classical microbiology

The classical microbiology workflow involves culturing a sample, observing colony morphology, and performing a single DNA analysis (e.g., SepsisTest BLAST) to identify the organism. The SepsisTest BLAST interface shows a FASTA sequence and a list of matches, including *Listeria ivanovii* T X985 and *Listeria seeligeri* DQ065844.

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

Metagenomics

The metagenomics workflow involves sequencing a mixed sample of 5 to 10,000 sequences per sample on a GS Junior sequencer. The resulting data is analyzed to produce a bacterial diversity profile, shown as a stacked bar chart. The chart displays the abundance of various bacterial groups at the class level, including Clostridia, Bacilli, Actinobacteria, Deltaproteobacteria, Unclassified, Erysipelotrichi, Alphaproteobacteria, Mollicutes, Epsilonproteobacteria, Betaproteobacteria, Fusobacteria, Verrucomicrobiae, Bacteroidetes, and Gammaproteobacteria.

1 analysis → 10,000 bacterial identifications

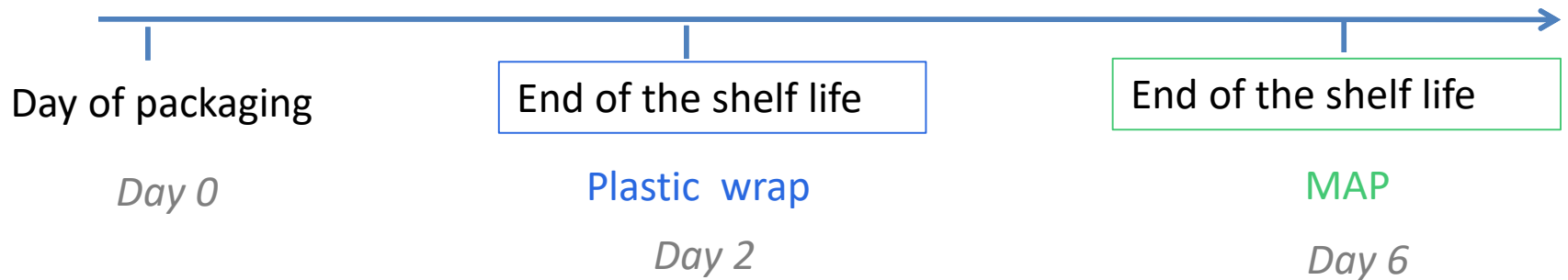
But are they the ones being sought ??

Analyzed samples:

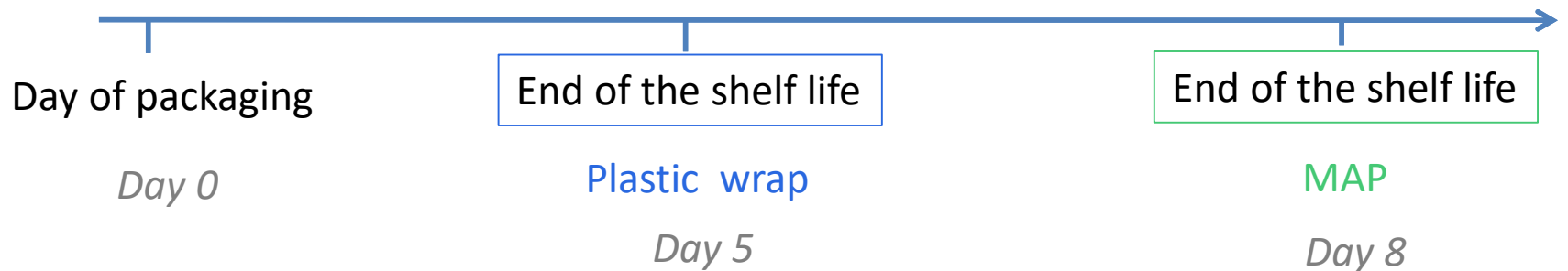
classical microbiology and metagenomic analysis



Seawater: Eglefin

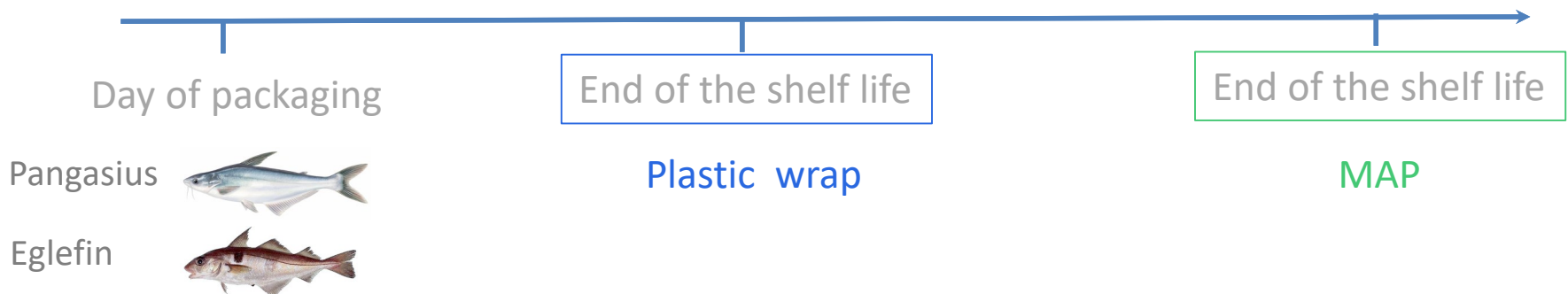


Freshwater: Pangasius



The aim of the study

Identification of flora involved in the fish from **freshwater** and **seawater**



➔ Validation of the shelf life

Results

With classical microbiology:

	PACC	LAB	PC	SA.	EC
Pangasius					
Day 0	3.66	<1	<2	1	1.3
under FW (5 days)	7.99	3.64	6.85	<1	3.38
under MAP (8 days)	7.74	6.48	6.32	<1	3.83
Haddock					
Day 0	4.3	1	4.04	<1	1.7
under FW (2 days)	5.96	1	5.95	<1	1.9
under MAP (6 days)	5.66	1.3	5.98	<1	3.26

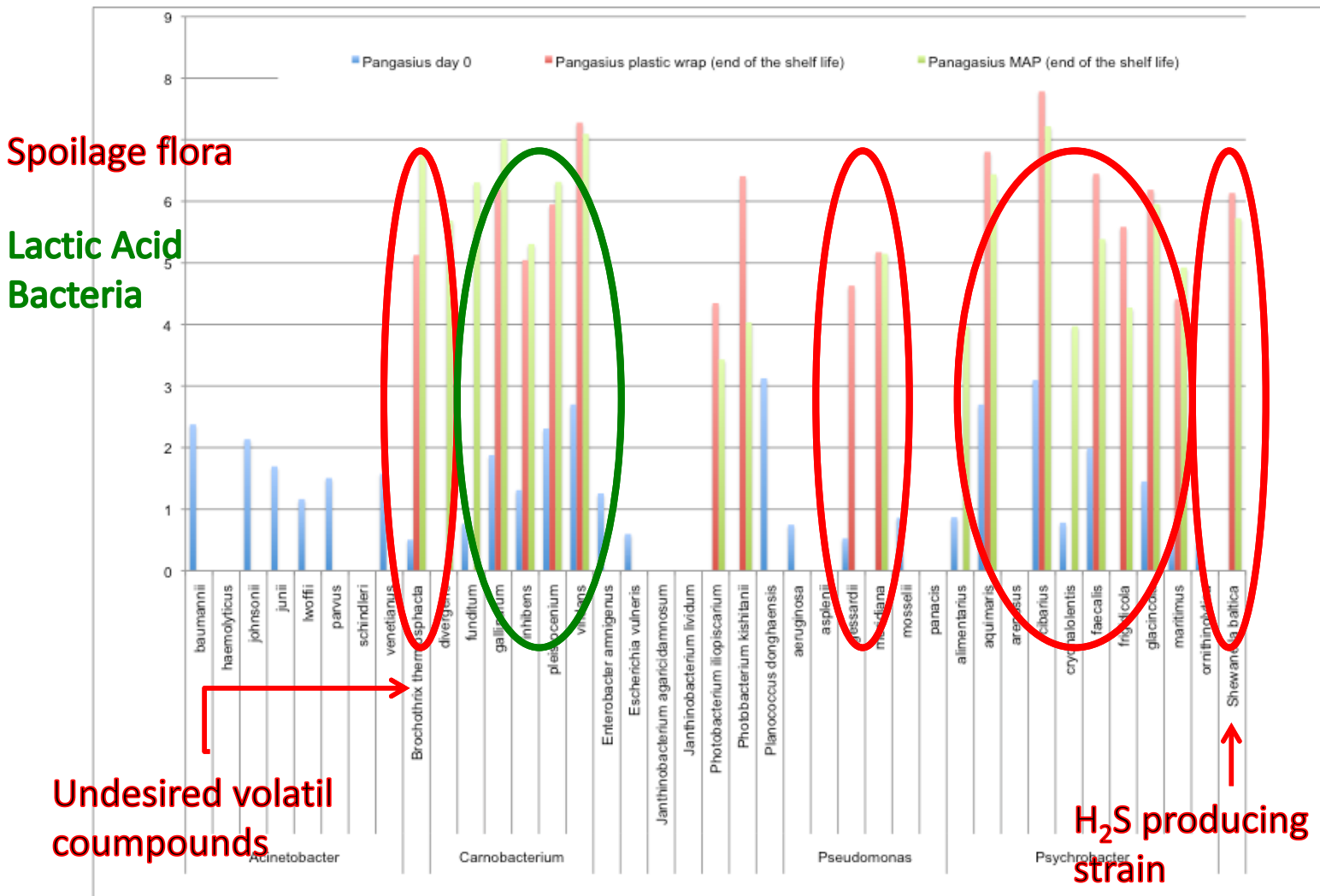
ACC (Psychotropic Aerobic colony counts), LAB (lactic acid bacteria), PC (*Pseudomonas* counts), SA (Staphylococcus aureus) and EC (*Enterobacteriaceae* counts). FW: Food Wrap; MAP: Modified Air Packaging

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 of the fish and packaging
- Relation between spoilage and the activity of some bacterial species

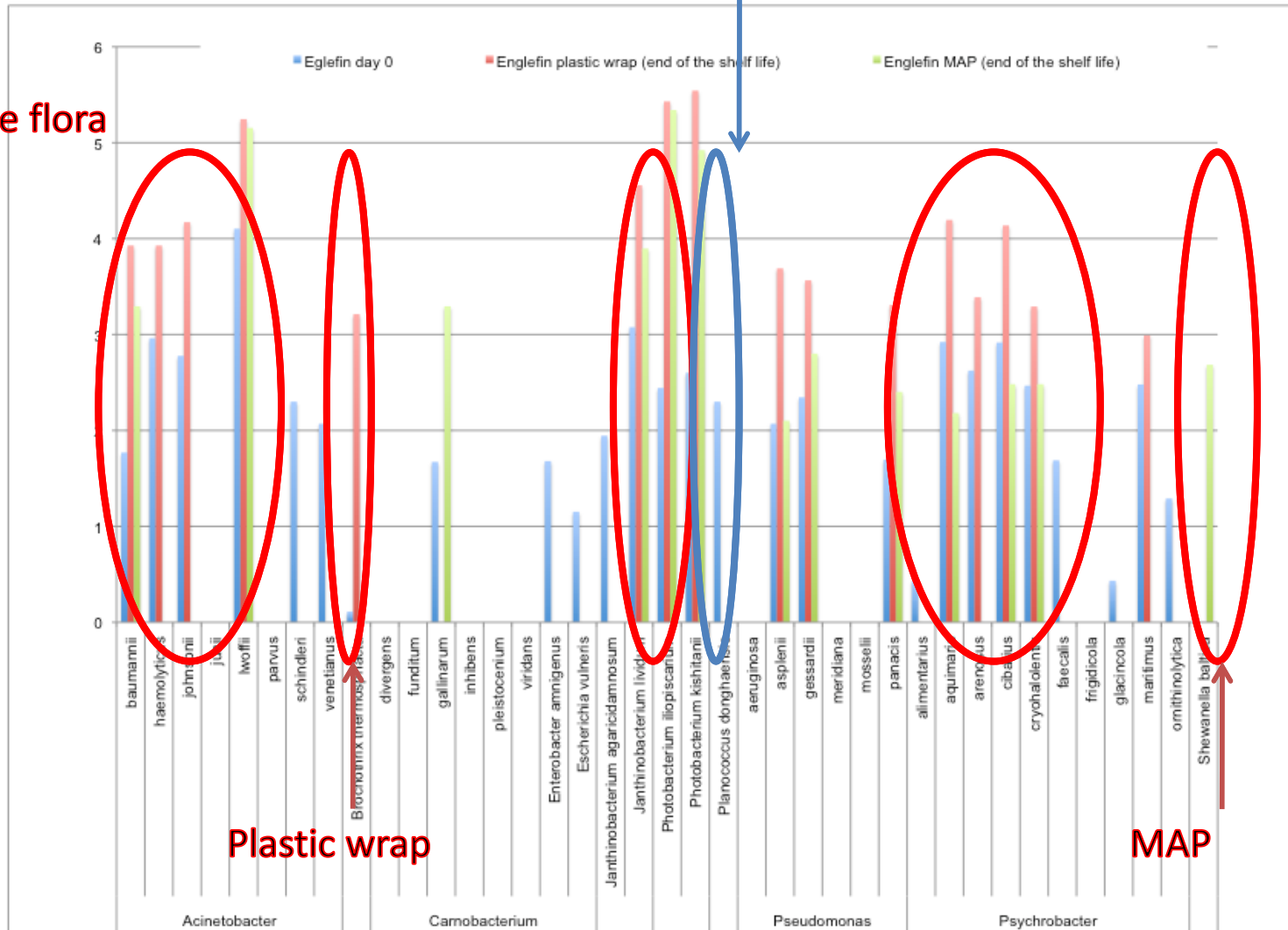
Pangasius (freshwater)



Haddock (seawater)

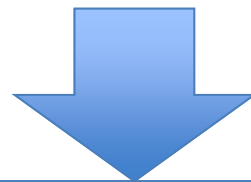
Freshness indicator

Spoilage flora



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 shelflife

- *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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