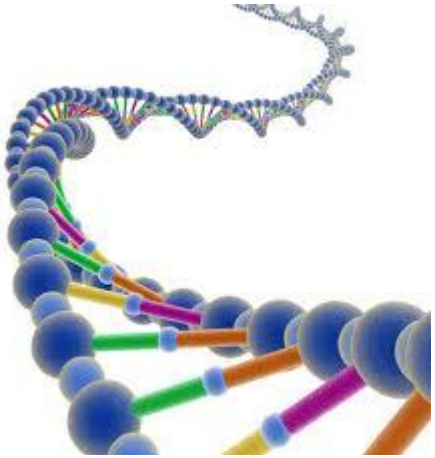
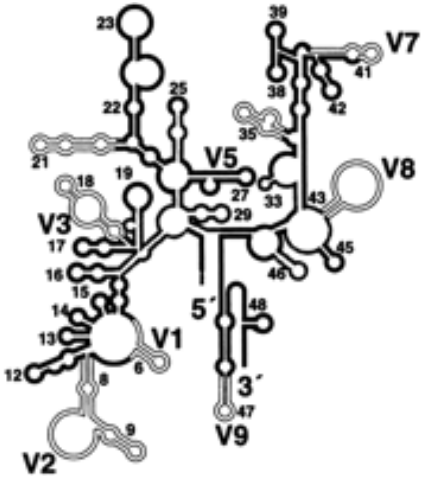
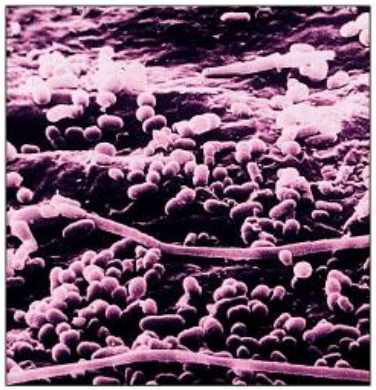
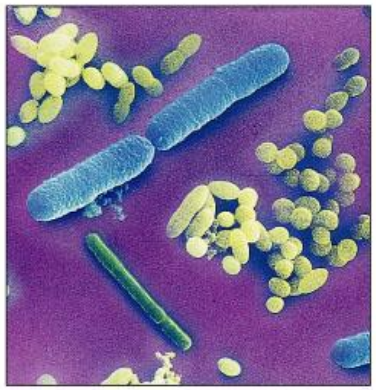




Nofima

Smart-label System - Microbial analyses





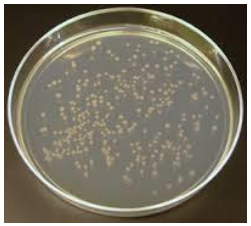
Birgitte Moen (birgitte.moen@nofima.no)

WP6 System and Acceptance Testing - Experiment Execution

- Testing of the Smart-Label System
  - Microbial analyses will be performed to verify the quality of the fish products and relate it to monitored temperature profile to external temperature logging
    - Traditional microbial plate counts
    - DNA based microbiota analyses
  - Rapid non-destructive freshness measurements

Nofima

Plate counts – total growth + selective medium

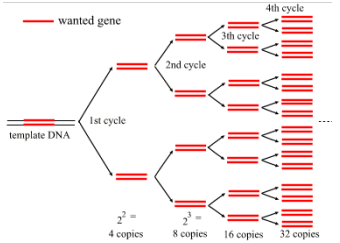


Collect samples

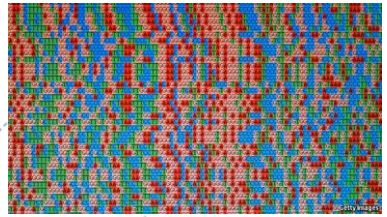
Homogenize the samples

Extract DNA

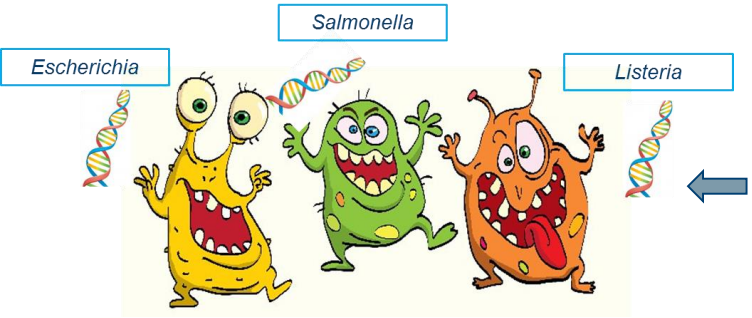
Copy “nametag”-part of the DNA



Sequence the DNA (= read the DNA code)



Search in databases



Mikrobiota analyses - Fingerprinting

- Can be used to construct a quick profile of the diversity of the microbiota
- The methods are based on DNA (or RNA) and are an alternative (or supplement) to cultivation based methods
  - Many bacteria do not grow under laboratory conditions
    - Need for selective mediums that often are not 100% selective
      - Growth is influenced by temperature, atmosphere and nutrients
- Parts of the DNA in bacteria can be used as a kind of **nametag**

- Next generation sequencing (NGS) is the preferred choice for fast and reliable microbiota analyses
  - **MiSeq** (Illumina) a bench top model of NGS

Nofima

MiSeq: Benefits and challenges

Benefits

- State of the art choice for microbiota analyses
- Fast and relatively cheap
- One can analyze several (hundreds) samples simultaneously
- No "*a priori*" knowledge about the bacteria is needed

Challenges

- qualitative and not quantitative (relative values of all bacteria present in the sample)
- Enables analysis on genus level, usually not species

This analysis is per date only applied as a research tool

Nofima

Examples from previous projects

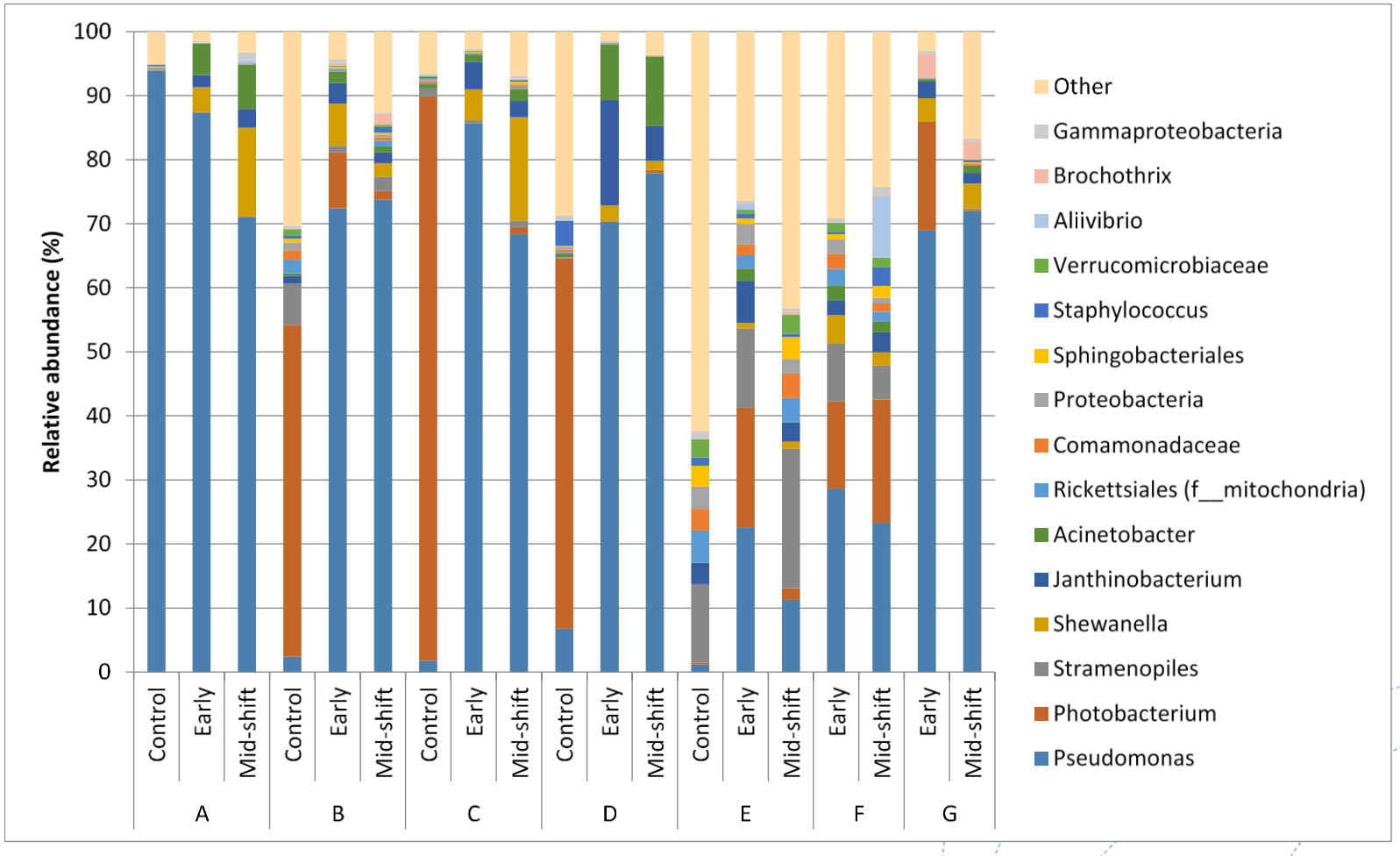
Hygiene and shelf life of salmon filets (FHF project)

- Aim: Evaluate the impact of hygiene, quality of raw material, storage conditions, microbial load and microbiota on quality and shelf life on ice storage salmon
  - Identify and characterize the microbiota on ice-stored salmon from different processing plants
  - Evaluate shelf life during spoilage with selected spoilage bacteria
    - Sensory analysis + consumer acceptance study

FHF

Nofima

Microbiota of ice stored salmon (10 days) from different processing plants



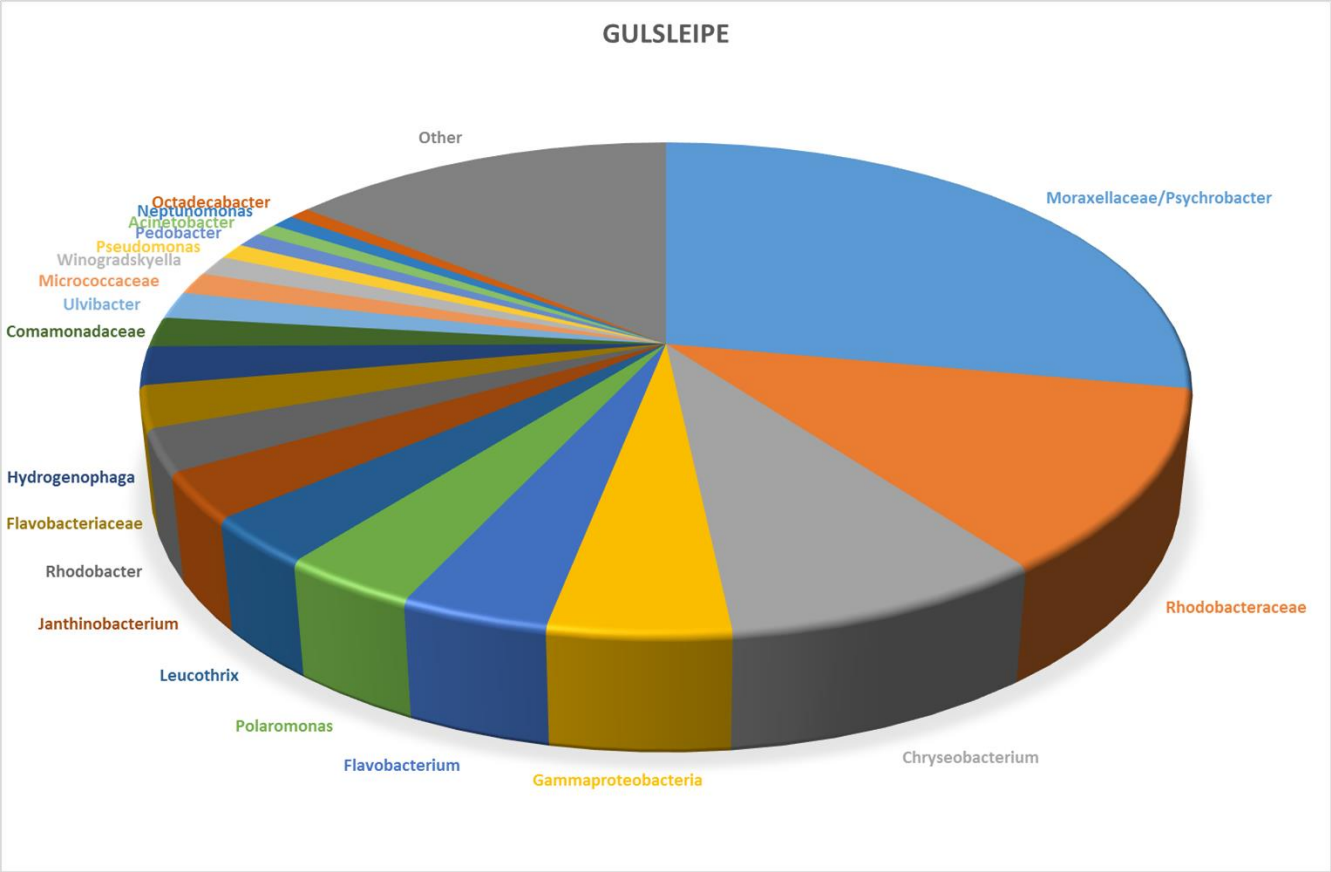
The controls were filleted by hand (best hygiene scenario) at Nofima

Results

- There were relatively large differences in bacterial load between the different processing plants – more or less the same dominating bacteria
- Salmon processed under optimal hygienic conditions was usually dominated by bacteria originating from the fish itself, e.g. *Photobacterium*
- During an industrial process the microbiota was influenced by the degree of contamination from equipment and water, and by temperature during processing
- Potential spoilage bacteria was isolated and used later in a controlled study simulating "optimal" and "sub-optimal" hygienic conditions
  - Consumer acceptance study
  - Sensory profiling
    - The different bacteria had different spoilage potential
      - *Photobacterium* approx. 10^7 cfu/g
      - *Pseudomonas* approx. 10^9 cfu/g
      - *Shewanella* approx. 10^8 - 10^9 cfu/g

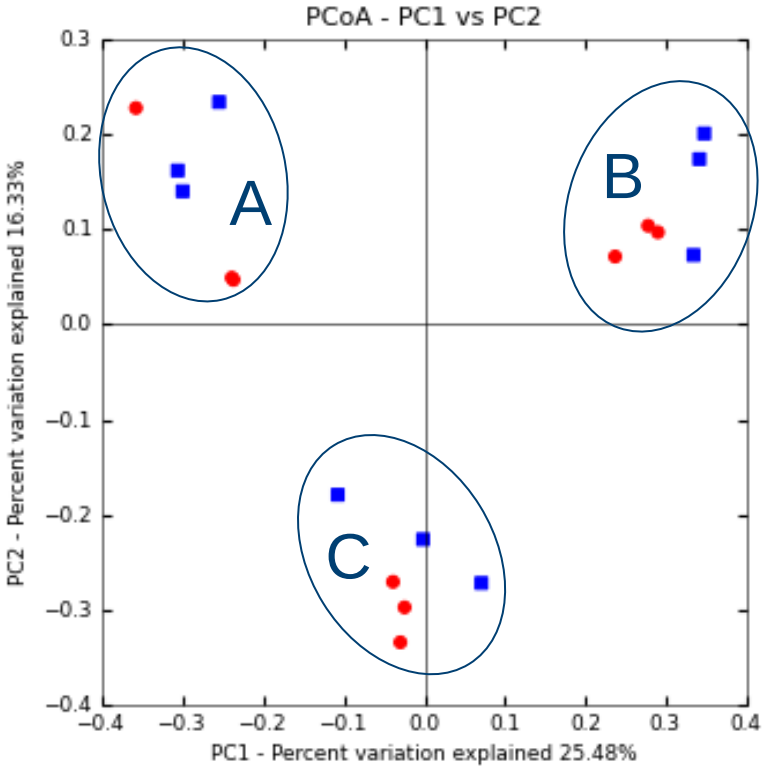
Microbiota of “gulsleipe”

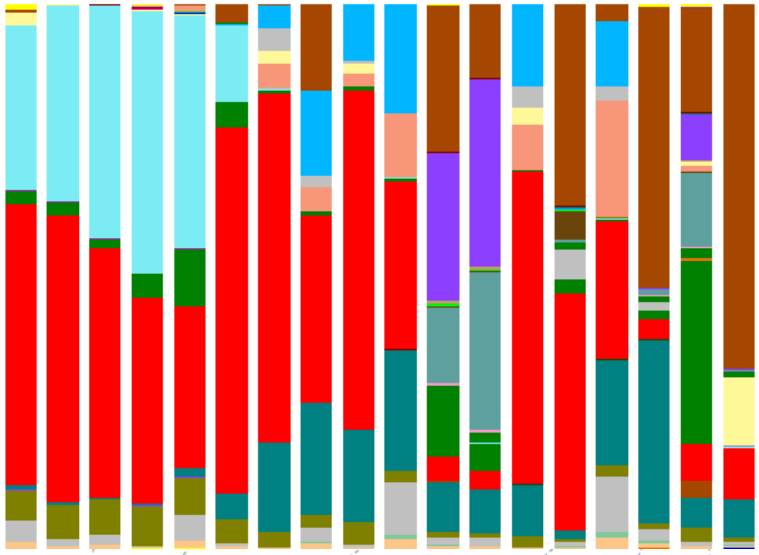
- During a project that aimed at looking at the hygienic conditions at different fish processing plants for white fish we performed DNA based microbiota analyses of “gulsleipe” samples



Fermented fish - «Rakfisk»

- Three different producers of Rakfisk
  - Different raw material and recipes (incl. salt and temperature)
  - Two sampling years (red and blue)





Conclusions

- The DNA based microbiota analysis is useful to get a compete overview of the bacteria present in the samples, and will in combination with traditional plate counts and non-destructive freshness measurements enable us to evaluate the Smart-Label System

Nofima