Rapid quantification of specific spoilage organisms (SSOs) in fish

using real-time PCR

TAFT, Copenhagen, 2009
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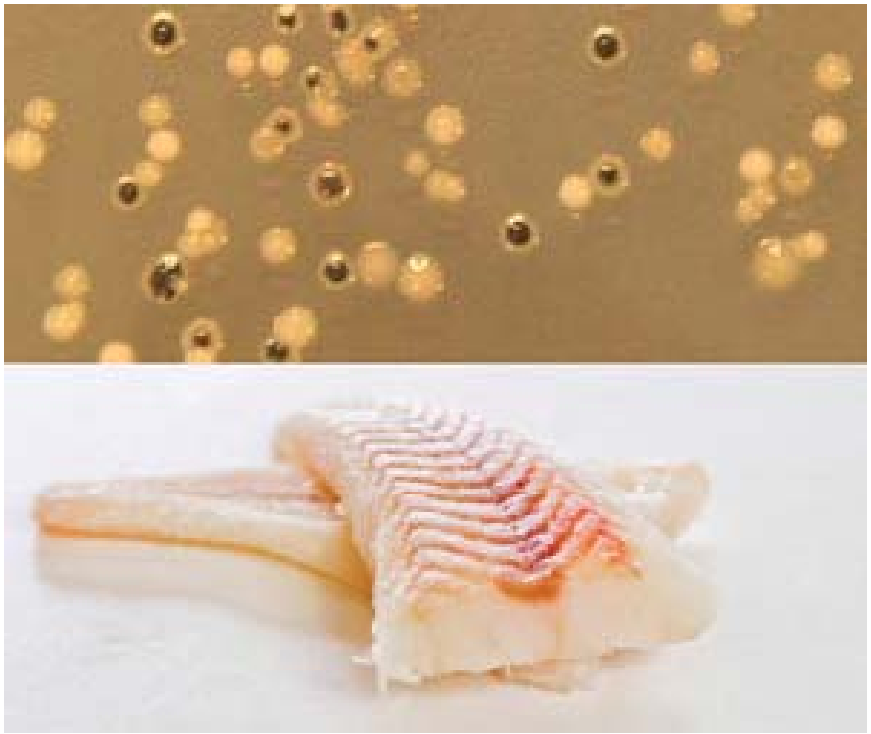
Presentation overview

- Background
  - The spoilage process
  - The specific spoilage organisms
- The technology
  - Real time PCR
- The method development
- Applications

Background

The spoilage process

- **The spoilage process of fish is composed of complex interactions between bacteria, the raw material itself and environmental factors**
- **Research activities have revealed the main bacterial spoilers which have been referred to as the specific spoilage organisms (SSO)**



Background

- **The microbiology of spoiling fish has been a matter of interest for scientists and the industry for decades**

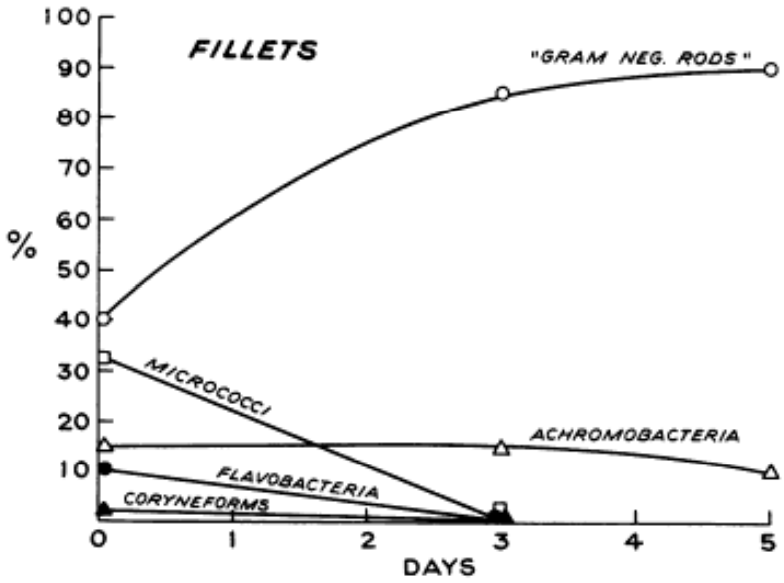


FIG. 4. *Changes in the composition of the aerobic bacterial flora of naturally contaminated fillets of sole during spoilage at 5.5 C. "Gram-negative rods" include all polarly flagellated, oxidase-positive asporogenous rods.*

Appl Microbiol. **1963** 11(5): 458–462.

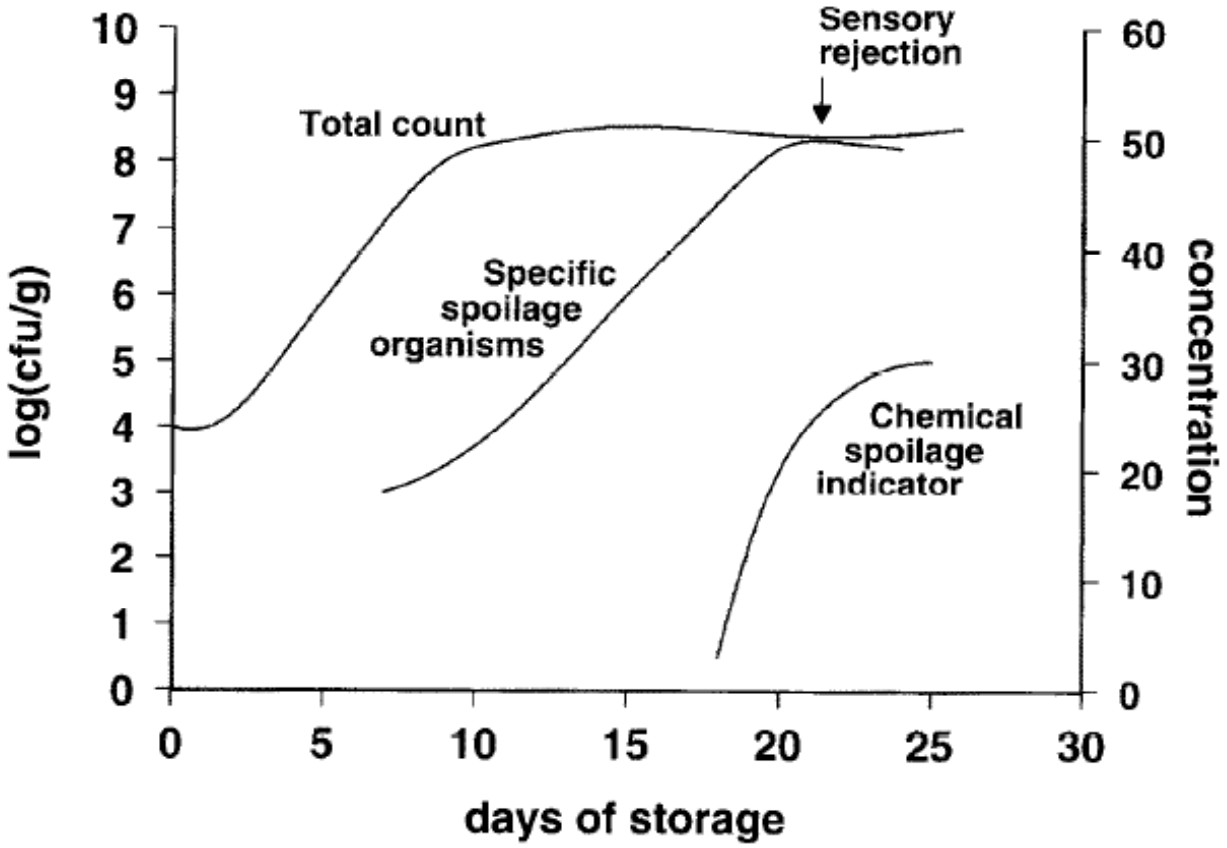
Bacteriology of Spoilage of Fish Muscle

I. Sterile Press Juice as a Suitable Experimental Medium

Peter Lerke, Ralph Adams, and Lionel Farber
Seafood Research Laboratory, San Francisco, California

Background

Number of bacterial species thrives in a spoiling fish but some of them are more active spoilers than others, contributing more to the staling smell and off-flavours of spoiled fish.



Which parameter is the most suitable marker to estimate product quality?

Fig. 1. Model of changes in total count (TVC), specific spoilage organisms (SSO) and chemical spoilage indices during chill storage of a fish product (modified from Huss et al., 1996).

Background

- **What bacteria are regarded as SSOs?**
  - **Can vary depending on the type of raw material, processing and storage conditions**
  - **Today the following indicator organisms are usually used to estimate product quality.**
    - *Pseudomonas* spp.
    - *Photobacterium phosphoreum*
    - H_2S producing microorganisms (*Shewanella*)

Existing quantification methods

Species	Method	# days
Pseudomonas	Cultivation on CFC agar at 22°C	3
Photobacterium	Malthus conductance method	2
Shewanella	Cultivation iron agar at 17°C	5

- **Cultivating the most important bacteria can give a good estimate on product quality during processing, transportation or storage**
- **The time frame however is to large to be able to use it for processing management purposes.**

The technology

- **In the EU project Chill-On, Wp2 is devoted to the development on rapid methods to detect and quantify undesirable bacteria in the fish processing chain (pathogens and spoilers)**

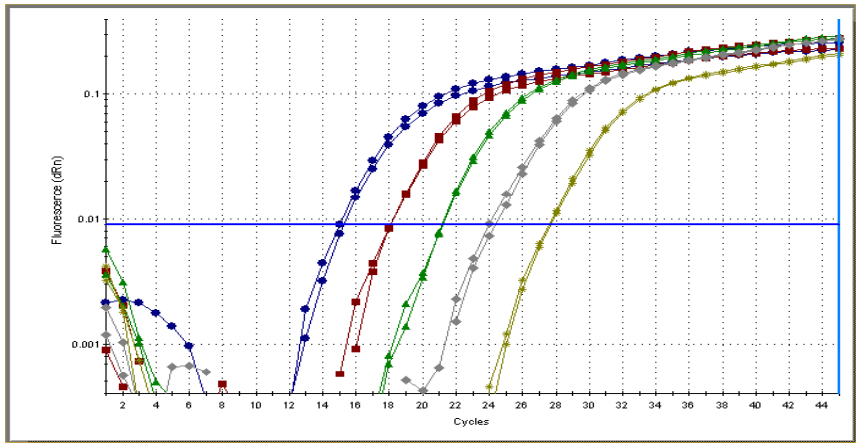
- **Our responsibility was the development on assay for enumeration of spoilage bacteria**

- **Real-time PCR was the method of choice**
  - **Rapid**
  - **Specific**
  - **Sensitive**

The technology

Real-time PCR

- Small part of the DNA molecule from the bacteria is amplified using DNA polymerase
- During amplification fluorogenic substances in the reaction emit light and is detected by the instrument
- The more bacteria present in a sample – the sooner the light is detected



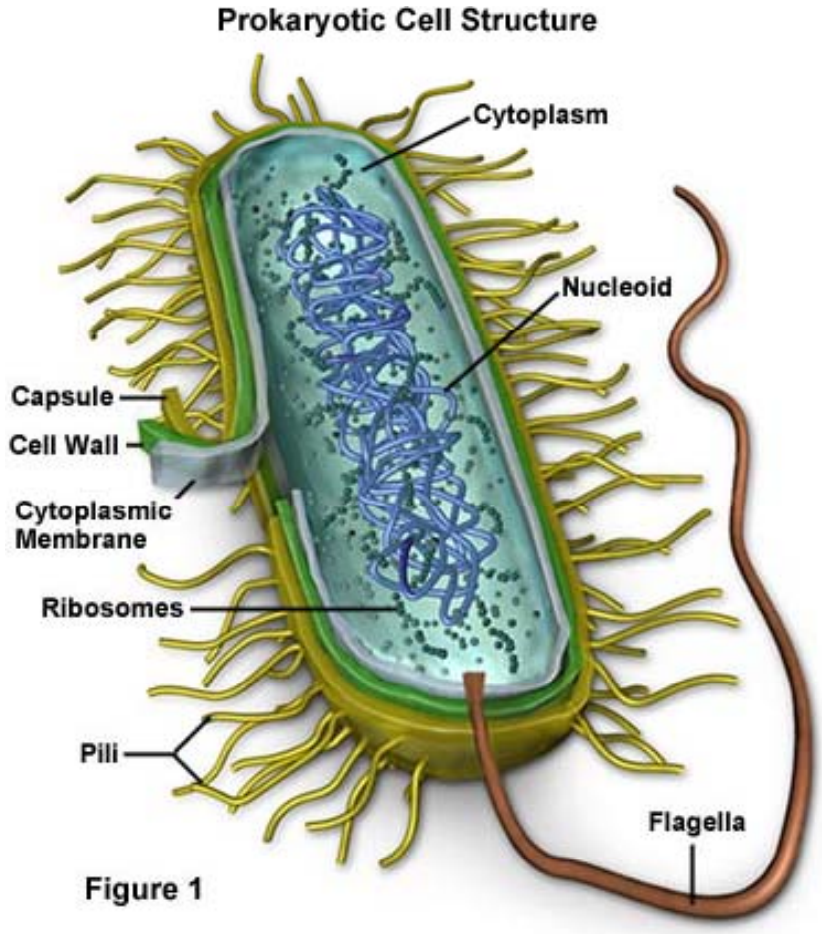


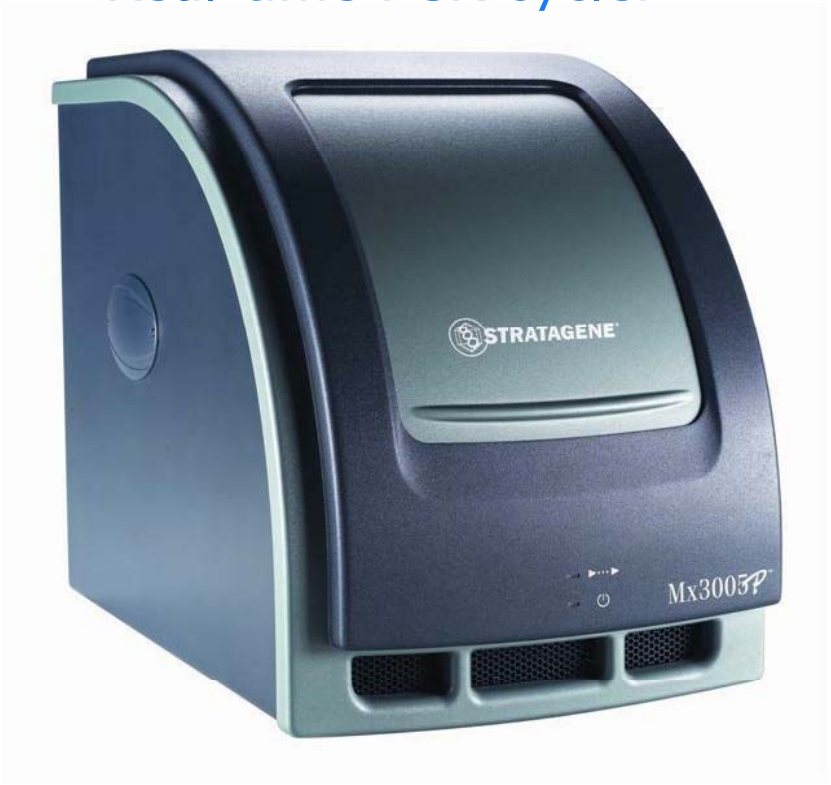
Figure 1

The technology

Required instrumentation:

Stomacher

Real-time PCR cycler



DNA extraction robot

Method development

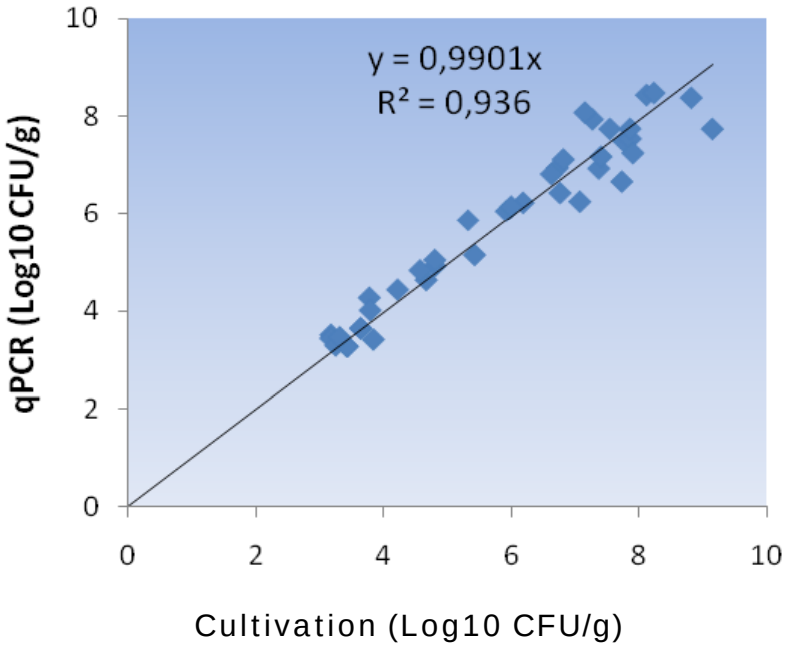
- **The key element of the assay lies therefore in the genetic elements of the bacteria. Using genetic information we were able to pinpoint unique areas in the genomes of the target organisms.**

- **The assay developmental phase included**
  - **Search of biomarkers**
  - **Testing of specificity and sensitivity**
  - **Optimization on reagents mixture**
  - **Optimization on sample preperation**
  - **Calibration of standards to cultivation**
  - **Testing of the assay in shelflife trials and comparison to existing methods**

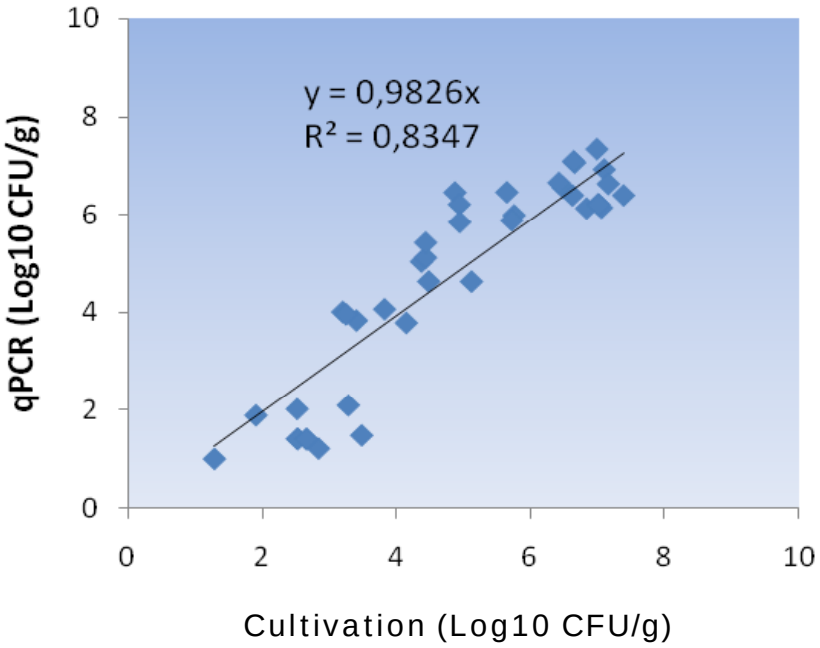
Method development

Comparative studies made on samples in a shelf life trial on cod, stored in various conditions (temperature, aerobic, MAP)

Pseudomonas

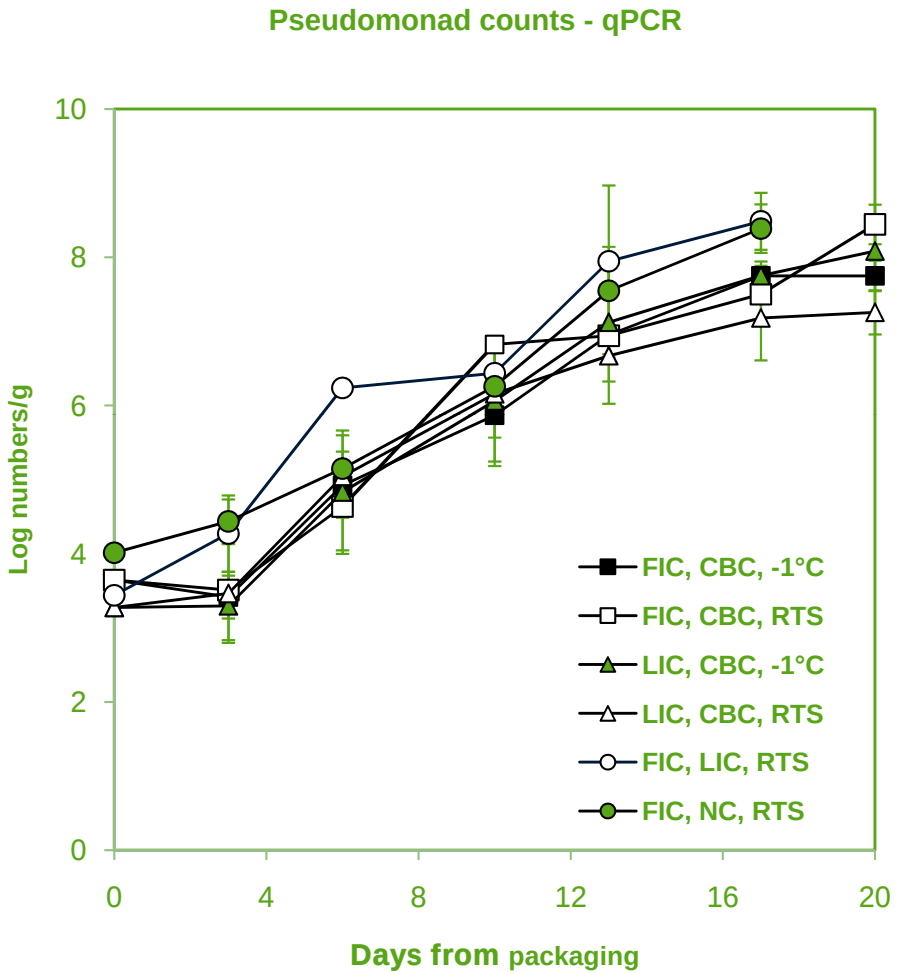


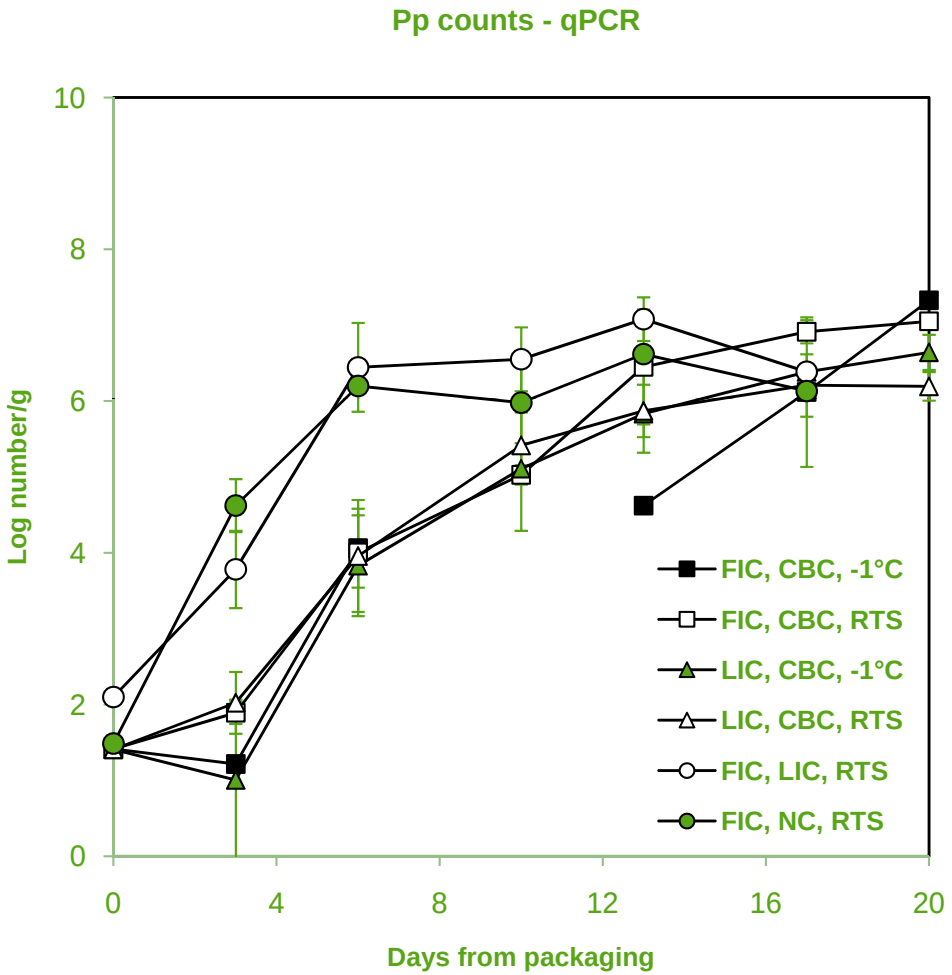
Photobacterium phosphoreum



Method development

Usability of the assay in storage trials
Assessment on assay performance



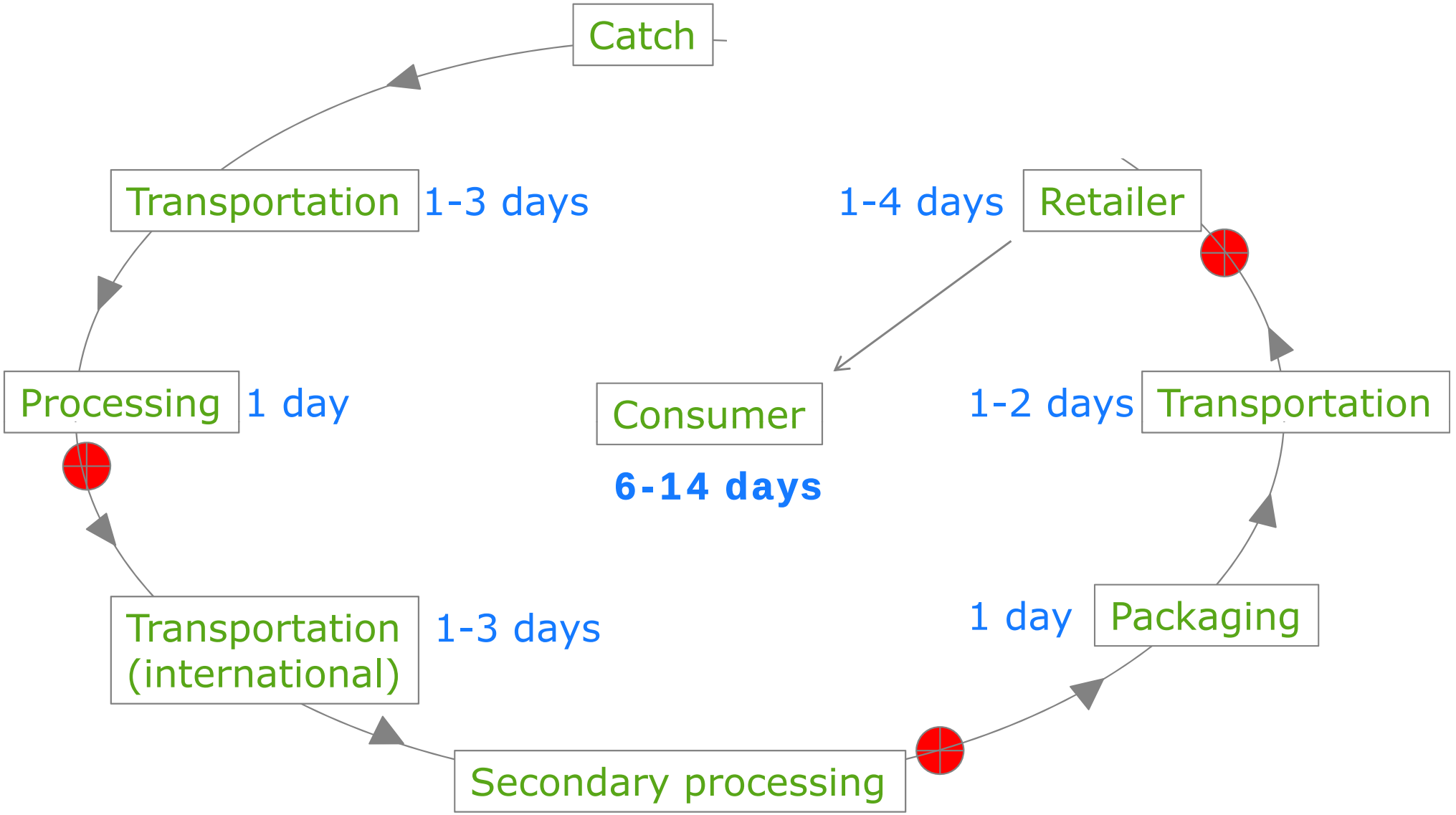


Time in analysis

Fish flesh minced and 25g diluted in 225mL buffer		**15 min**
DNA extraction		**90 min**
PCR analysis		**120 min**
Reults analysis		**15 min**
	Total	**4 hours**

=> Results within **5 hours**

Applications in the supplied chain



Applications

- **For producers – quality control management**
- **For buyers – quality control management**
- **Implementation into quality control programs e.g. HACCP**
- **Implementation with microbiological models, prediction of remaining shelf life**