# Differences in the dominant microbiota present on various growth media applied in fish analysis

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## Introduction & Aims

Microbiological (quality) control of fish is necessary for the determination of the remaining shelf-life and important for food safety. At present, the enumeration of microbiota on food is often analysed by a total viable count on Plate Count Agar (PCA), such as recommended by the International Organisation of Standardization (ISO). If the total viable count exceeds $10^7$cfu/g, the fish is no longer consumable. For research matters however, fish microbiology is mainly determined on salt-containing media such as Marine agar (MA), Long and Hammer (LH) and Iron agar (IA), since several marine micro-organisms are halophilic.(1,2)

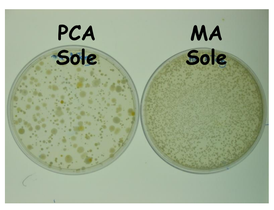
Research has revealed that only a small fraction of the micro-organisms present on fish tend to cause spoilage, namely the specific spoilage organisms (SSOs). *Pseudomonas* sp., *Shewanella putrefaciens*, *S. baltica* and *Photobacterium phosphoreum* are examples of commonly found specific spoilage organisms of marine fish.(3,4,5,6) In this study, a comparison on total count between PCA and marine media was made based on traditional microbiological techniques as well as by DGGE-analysis. Additionally, the micro-organisms specifically not growing on one of those media were identified.

## Material & Methods


- The microbiota of 11 fish species was monitored during ice storage. The fish samples were collected from the distribution centre of a large supermarket and were analysed during regular time intervals on 4 different media:
  - Plate CountAgar (PCA,Oxoid)
  - Marine Agar (MA,Difco)
  - Long and Hammer (LH)(7)
  - Lyngby Iron Agar (IA)
- After incubation, the colony growth on the different media was compared using a replication technique.
- 125 non-growing colonies on one of the media at 7 days of ice storage, were collected, purified and DNA was extracted based on a modified Flamm (1984) extraction followed by ($GTG_5$) rep-PCR clustering and 16S rRNA gene sequencing (± 700bp)
- Identification for 24 strains was done by comparison to the EZTAXON database(8)
- Analysis of the total bacterial count by DGGE – V3 region

## Results & Discussion

### Microbiological analysis




- For all 11 fish species an **underestimation on PCA** was detected. The **total count on PCA** was **0.5 log to 1.8 log lower** than on marine media, depending on the fish sample, fish species and the period of ice storage. A difference of 1 log is in accordance with literature.(1,2)
- The **highest difference** occurred between PCA and LH medium, the smallest between LH and MA.
- After replication from the marine media (LH) to PCA, it was shown that a lot of colonies (up to 90% for some fish species) were not able to grow on PCA (results not shown).

### Identification of the bacterial isolates

- After rep-PCR clustering, **24 strains from time interval 2** (after ice storage) were selected for identification by 16S rRNA sequence analysis.
- **14 different genera/species** were identified (table 2).
- Based on the number of identical rep-profile, ***Shewanella* sp.** and ***Pseudoalteromonas* sp.** are very common on fish during ice storage and will not be detected by total count analysis on PCA. Also the other SSO ***Photobacterium* sp.** and ***Vibrio* sp.** were **unable to grow on PCA.**

| | Grows not on | N° of identical isolates based on the $GTG_5$ profile | Nearest phylogenetic neighbour(s) by 16S rRNA sequence analysis | Found in fish species |
|---|---|---|---|---|
| 1 | MA | 5 | *Flavobacterium* sp. | Seabream, anglerfish, plaice |
| 2 | MA | 1 | *Janthinobacterium* sp. | Cod |
| 3 | MA | 4 | *Pseudomonas* sp. | Anglerfish, seabream |
| 4 | IA | 2 | *Brochothrix thermosphacta* | Seabream, sole |
| 5 | PCA | 50 | *Shewanella* sp. | All fish except ray |
| 6 | PCA | 1 | *Shewanella baltica* | Cod |
| 7 | PCA | 1 | *Vibrio* sp. | Sole |
| 8 | PCA | 3 | *Photobacterium* sp. | Sole, plaice, whiting |
| 9 | PCA | 4 | *Photobacterium phosphoreum* | Cod, salmon, whiting |
| 10 | PCA | 33 | *Pseudoalteromonas* sp. | All fish except cod |
| 11 | PCA | 7 | *Psychrobacter* sp. | Anglerfish, seabass, seabream, *Pangasius*, plaice |
| 12 | MA,LH, IA | 4 | *Psychrobacter* sp. | Anglerfish, seabass, cod |
| 13 | MA,LH,IA | 1 | *Acetinobacter* sp. | Seabass |
| 14 | MA,LH,IA | 1 | *Pseudomonas fragi* | Seabass |

**Table 2:**
14 different identified strains with their number of identical isolates, the media on which they are inable to grow and the fish species they were selected from.

### DGGE analysis

- A DGGE-analysis was performed on plateswabs of all media
- As seen in figure 1, the **variation in bandpattern between PCA** (P) and the **other media** (M,L,I) differed from each other at both sampling periods, even for *Pangasius* (fresh/brackish water fish).
- The patterns observed from the **marine media** (M,L,I) were often **similar**.
- **Internal markers** were used to identify some of the abundant genera. This was possible only for a few genera (eg. *Psychrobacter* and *Pseudoalteromonas*). All pure strains of these 2 genera gave a pattern in which the colored band used for identification was present.
  For several other strains, this was not possible since a **complex band pattern** was obtained from a **pure strain** (eg fig 1, Sh and Ps).
- A **variation in number of genera** growing on the different media **could not be made** since some species tend to have a complex band pattern by using the V3 region of the 16S rRNA gene.

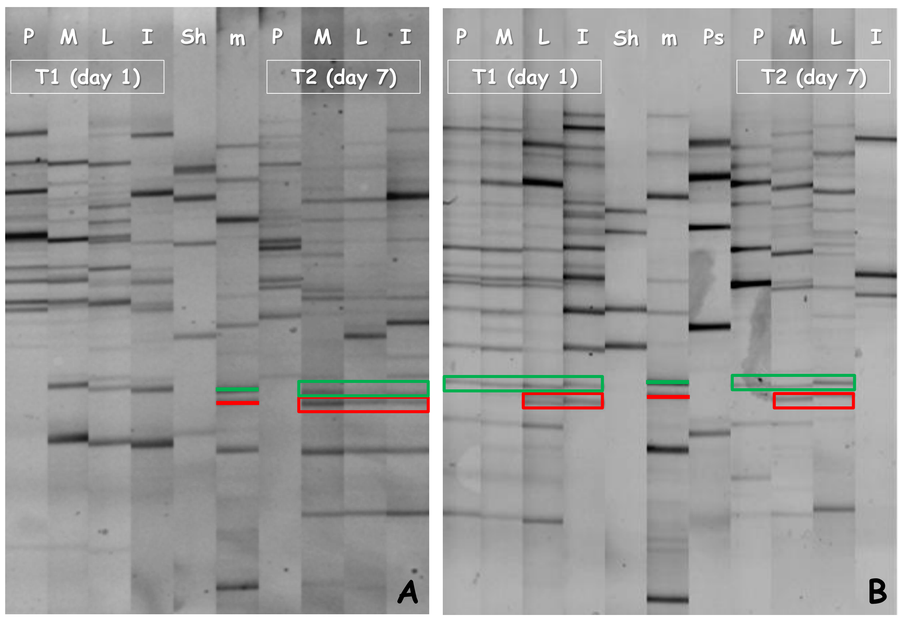



**Fig 1 A & B:**
DGGE – V3 analysis of a swab of all media P (PCA), M (MA), L (LH), I (IA) of Pangasius (A) and mackerel (B) at the day of arrival (T1) and after 7 days of ice storage (T2). Internal markers were included: m (*Psychrobacter*, *Pseudoalteromonas*), Sh (pure *Shewanella* strain) and Ps (pure *Pseudomonas* strain)

## Conclusions

**By the use of one single medium in the microbiological analysis of fish, a lot of micro-organisms will not be detected. In this study it was shown that some specific spoilage organisms such as *Shewanella* sp. and *Photobacterium* sp. and other marine micro-organisms growing during ice-storage (*Pseudoalteromonas* sp., *Vibrio* sp.) could not be detected on PCA which is possibly caused by the lack of salts in this medium. On the other hand, some micro-organisms present on fish during storage could not grow on (one of) the marine media, such as *Flavobacterium, Acetinobacter* sp. and some *Pseudomonas* species.**

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