# Spoilage micro-biota of sea-bream stored in ice identified by phenotypic and 16S rRNA gene analysis

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## Aims

To investigate, using molecular and classical approach, the synthesis of initial and spoilage micro-biota of aquacultured sea-bream (*Sparus aurata*) stored in ice

## Methodology

- DNA extraction from 0.2-0.6 g of flesh by using the Ultra Clean Soil DNA kit (MoBio, USA).
- PCR amplification with the bacterial universal primers GM3 (5'-AGAGTTTGATCMTGGC-3') and GM4 (5'-TACCTTGTTACGACTT-3').
- After PCR product purification (Montage kit, Millipore, USA), cloning was performed by using the TOPO TA Cloning kit (Invitrogen, USA
- Sequencing was performed by the BigDye Terminator technique (Macrogen, Korea) of the first ca. 850 bp of the 16S rRNA gene
- Sequences' similarity was checked with CLUSTALW and their closest relatives were found from GenBank (www.ncbi.nlm.nih.gov)
- Phenotypic determination of colonies grown on TSA was carried out using conventional tests

## Results

Initially, the fish flesh hosted a very low abundance bacterial community, practically related only to *Acinetobacter* sp., probably originating from seawater. On the contrary, at the end of shelf-life *Aeromonas salmonicida* dominated, followed by three *Pseudomonas* sp. phylotypes.

**Table 1.** Percentage (%) of isolates grown on TSA

| | Initial micro-biota | Spoilage micro-biota |
|---|---|---|
| ***Shewanella putrefaciens*** | 28.6 | 25.0 |
| ***Pseudomonas fluorescens*** | 26.0 | 75.0 |
| ***Flavobacterium* sp.** | 10.4 | |
| ***Acinetobacter* sp.** | 35.1 | |

## Conclusions

Initial diversity was very low, with *Acinetobacter* sp. being the dominant microorganism. *Acinetobacter* sp. is common phylotype in sea environment. Spoilage micro-biota comprised phylotypes that have been identified by other researches using culture techniques. However, *Aeromonas* sp. has not been reported as predominant spoilage micro-biota of sea-bream.

## Significance of study

Coupling of molecular and classical methodologies better reveal the micro-biota of sea-bream during storage, providing us with valuable information on spoilage and safety aspects.

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**Table 2.** The most dominant phylotypes of initial and spoilage micro-biota determined using molecular approach

| Phylotype | Frequency in clone library | Closest relative, Similarity (%) [GenBank No.] | Habitat | Reference |
|---|---|---|---|---|
| **Initial micro-biota** | | | | |
| FP_3F-M13F | 59.2% | Uncultured *Acinetobacter*-like clone PEACE2006/173_P3 (99.9%), [EU394575] | Particles from the water column of the Northern Bay of Biscay, pelagic | Unpublished |
| FP_2F-M13F | 34.7% | Uncultured *Acinetobacter* sp. clone IODP_305_1309D_250_21 (99.9%), [HQ379140] | Gabbroic central dome of the Atlantis Massif Hole 1309D (deep subsurface) | Mason et al. (2010) First investigation of the microbiology of the deepest layer of ocean crust. PLoS ONE 5 (11), E15399. |
| **Spoilage micro-biota** | | | | |
| B3 | 28.6% | *Aeromonas salmonicida* strain CIP 57.50, (99.8%) [FJ936134] | | Minana-Galbis et al. (2010) Int. J. Syst. Evol. Microbiol. 60: 715-717 |
| B2 | 14.3% | *Pseudomonas* sp. DD1 (100%), [HQ113379] | Digestive tract | Martin-Creuzburg et al. (2011). FEMS Microbiol. Ecol. 76: 592-601 |
| B1 | 9.5% | *Pseudomonas putida* PhyCEm-187, (99.7%), [AM921634] | *Lolium perenne* rhizosphere | Unpublished |
| B5 | 9.5% | Uncultured bacterium clone A8 (99.5%), [GQ422761] | Raw pork | Unpublished |
| B6 | 9.5% | *Shewanella* sp. S2 (98.6%), [FJ589033] | Sea sediment | Unpublished |
| B13 | 9.5% | *Pseudomonas fluorescens* strain LMG 14577, (99.6%), [GU198122] | Polluted seawater | Unpublished |