Use of 16S rRNA gene analysis for the identification of dominant microbiota in sea bream fillets stored at various conditions

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Introduction

The use of 16S rRNA gene diversity analysis is a powerful tool for studying microbial diversity in practically every natural or artificial habitat. The aim of this study was the investigation of initial and spoilage microbiota of sea bream (*Sparus aurata*) fillets stored under air and MAP using 16S rRNA gene analysis.

Methodology

Characterization of the initial and spoilage microbial diversity of sea bream fillets stored under air and Modified Atmosphere Packaging (MAP, CO_2: 60%, O_2: 10%, N_2: 30%) at 0 and 5^{o}C was investigated by using pure culture technique and 16S rRNA gene analysis.

- Isolated colonies from TSA of initial (42 colonies) and spoilage microbiota of sea bream fillets stored under air (38 & 32) and MAP (37 & 36) at 0 and 5^{o}C respectively, were grouped according to their morphological characteristics and further purified on new TSA plates.
- The isolates were identified using commercially available biochemical identification API kits (BioMérieux, France).
- DNA from isolates was extracted and PCR was performed with universal primers 27fBAC (5'-AGAGTTTGATCMTGGCTCAG-3') and 907rBAC (5'-CCCGTCAATTCCTTTGAGTTT-3') with 33 cycles
- Sequencing was performed by the BigDye Terminator technique, and the sequences' similarity was checked with CLUSTALW and their closest relatives were found from GenBank (www.ncbi.nlm.nih.gov)

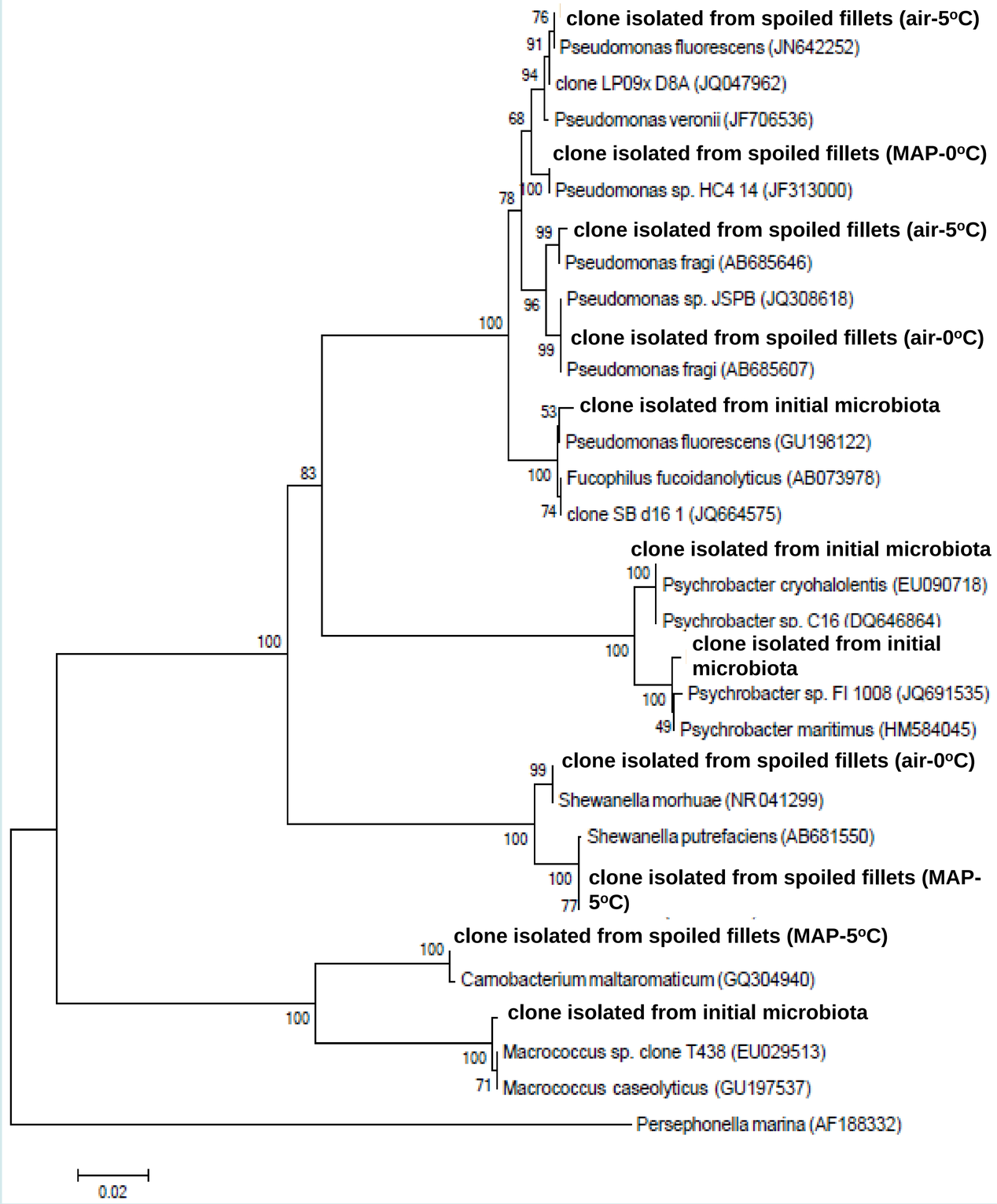
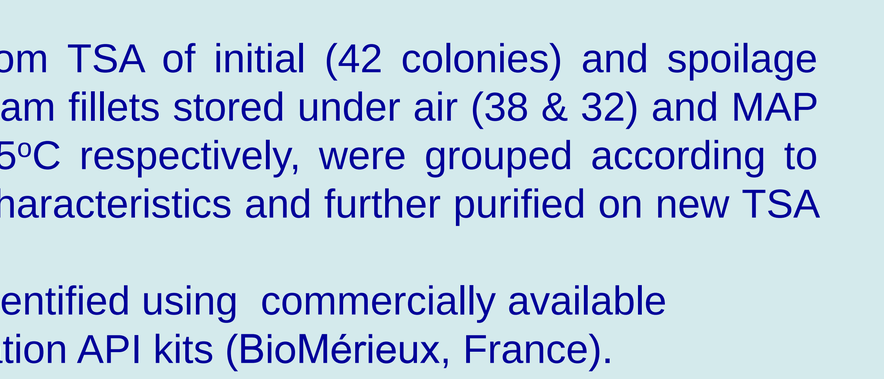


Figure 2. Phylogenetic tree of the PCR-amplified bacterial 16S rRNA gene phylotypes (in bold) (ca. 900 bp) from the sea bream initially and at the end of shelf life of fillets under air and MAP at 0 and 5^{o}C, based on the neighbor-joining method as determined by distance using Kimura's two-parameter correction.

Results

Sea bream micro-biota by pure culture technique



and…by 16S rRNA gene analysis

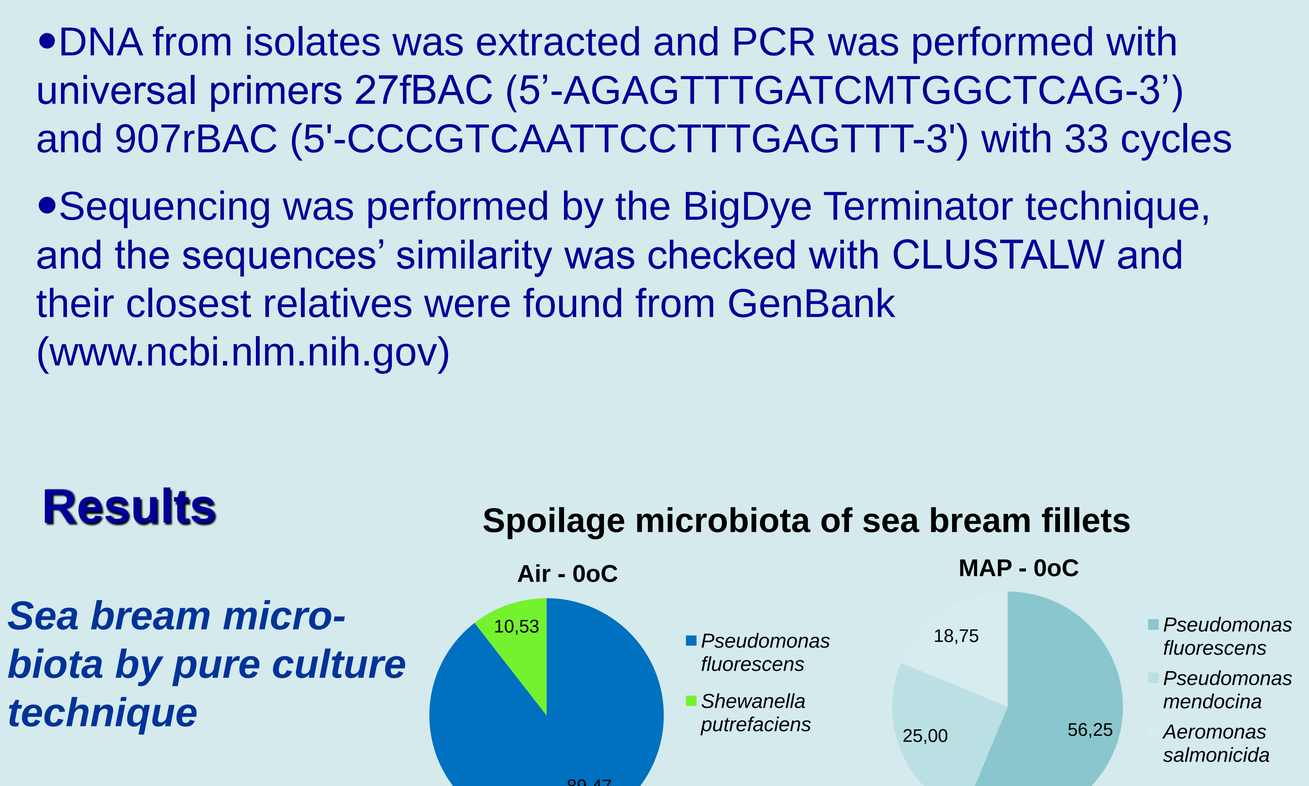


Figure 1. Percentage (%) of isolates grown on TSA determined by pure culture technique and 16S rRNA gene analysis

Conclusions

Our results ascribe the 16S rRNA based approach complementary to the traditional methodology by assisting the precise identification of most of the bacteria, in contrast to phenotypic methods. Furthermore molecular approach reveals genus and species that escape the traditional approach, like ***Psychrobacter*** and ***Carnobacterium*** in our case.

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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