DEVELOPMENT OF HIGH-RESOLUTION MOLECULAR TECHNOLOGIES TO INVESTIGATE ECOSYSTEM CHANGES IN THE SOUTHERN EUROPEAN SEAS: THE PROTOTYPING OF DNA CHIPS TO IDENTIFY INVERTEBRATE PREY SPECIES IN MARINE FOOD WEBS OF ADRIATIC SEA

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Abstract

A invertebrate DNA microarray targeting ten species common in the Mediterranean was developed within the framework of the FP6 STREP FISH& CHIPS. Based on sequence variation of the 16S rDNA gene, we designed species-specific probes that were used to construct a DNA chip prototype for the identification of invertebrate (i.e. molluscans and crustaceans) preys of demersal fishes in the Adriatic Sea. The constructed invertebrate DNA chip represents a technological advance towards the wide use of rapid and high-resolution molecular tools to assess structure and changes of marine ecosystems such as the monitoring of invasive species and of re-emerging pathogens in ballast waters.

Keywords : Biotechnologies, Adriatic Sea, Food Webs, Species Introduction, Zoobenthos.

The understanding of trophic interactions among organisms and in the marine ecosystem is crucial for sustainable management of fishery resources and for conservation of marine biotas [1, 2]. Unfortunately, the analysis of gut contents carried out using the traditional analytical approach of morphological identification is time-consuming and requires highly-specific taxonomic expertise. Here, within the framework of the FP6-STREP FISH& CHIPS, we aim to develop a prototype DNA chip targeting invertebrate species (INVCHIP) that are common preys of demersal fish species in the Mediterranean Sea [3].

Ten invertebrate species belonging to molluscans and crustaceans widely distributed in the entire Mediterranean have been sampled in the Adriatic Sea. A 500 bp PCR-amplified 16S rDNA fragment of at least three individuals per species was sequenced using universal or taxon-specific primers. The capture probe design was performed through multiple alignments of DNA sequences using the programmes WinProbe and GeneDoc. Sequences of phylogenetically related species were retrieved from the NCBI nucleotide data bases and used for probe design. Chip configuration, probe immobilisation and hybridisation experiments have been done according to available protocols. Positive and negative controls were added to each microarray.

An INVCHIP-1 was realised including 6 target species (Mollusca: *Illex coindetti*, *Eledone cirrhosa*, Crustacea: *Meganychtiphanes norvegica*, P *achigrapsus marmoratus*, *Liocarcinus depurator*, *Liocarcinus vernalis*). 14 probes were designed and tested with subsequent hybridisation experiments. Capture probes gave different results: four oligonucleotide probes were specific for their target DNA (one for each *I. coindetti*, *E. cirrhosa*, *P. marmoratus*, *L. vernalis*); one probe hybridised the target species *L. depurator* but also the closely related species *L. vernalis*; nine probes did not bind the target DNA., Preliminary experiments carried out under different experimental conditions (i.e. decreased Tm and increased hybridisation time) allowed increasing of specificity and sensitivity for some probes and target species. The realisation of a INVCHIP-2 (including Mollusca: *Lentidium mediterraneum*, *Chamalea gallina*; Crustacea: *Alpheus glaber*, *Goneplax rhomboides*) is now in progress through probe design and chip configuration steps. Hybridisation experiments are scheduled for the end of 2006.

The developed INVCHIP represents a relevant technological advance towards the wide use of rapid and high-resolution molecular tools to assess structure and changes of marine ecosystems. Beside the investigation of marine food webs, DNA microchips coupled with a metagenomic analytical approach can effectively contribute to the monitoring of invasive species (either as eggs or larval stages) and re-emerging pathogens in ballast waters which are reversed daily in the Southern European Seas [4-6].

References

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