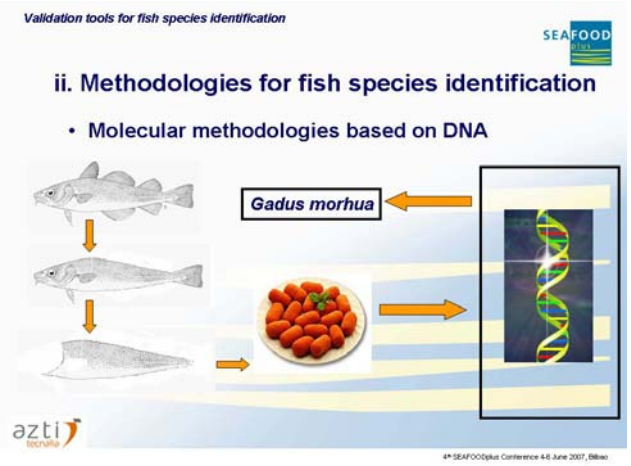
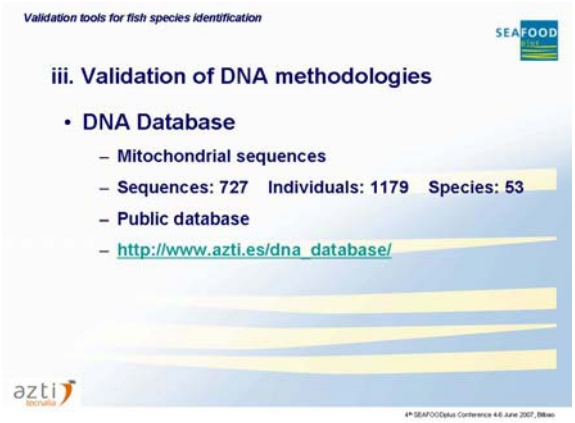
Validation tools for fish species identification

Miguel Angel Pardo
Food Research Division, AZTI Instituto Tecnológico Pesquero y Alimentario, Spain





During the last years several fish species identification methodologies based on DNA technology were developed to fulfil the European Union regulations (EC No 104/2000). This regulation stresses the necessity of labelling the seafood products with the scientific name to assure the traceability system through the whole chain. Most of these methodologies are based on the amplification by PCR of a specific DNA marker from the whole genome. Mitochondrial genome is so far the most common source of DNA markers for fish species identification and in particular the cytochrome *b* gene. Actually, the establishment of a mitochondrial DNA Sequences Database was one of the main objectives of the project VALID. This Access Database has been developed to be a user-friendly tool to validate DNA analysis methodologies by PCR FINS. In addition, it is a source of DNA sequences that can be used to design innovative methodologies for species identification in seafood products. Database was also accessible by internet.

In addition, a pool of certificated DNA standards was developed as a tool to validate DNA analysis techniques and to be used as internal control of the PCR reaction. To date, the validation of the DNA methodologies was performed using genomic DNA isolated from reference tissue samples which are not standard. The use of plasmidic standards, with the same characteristics and composition, ensure the standardization of measurements and procedures, and thus allowing the comparison of results obtained in different laboratories. Moreover, a precisely quantified PCR standard provides valuable information about true positive/negative results, the optimum PCR parameter and the estimation of the initial amount of DNA template.

The innovative set of standards was designed with the complete mitochondrial cytochrome *b* gene from different fish species of gadoids, tunas, hakes and anchovies with the corresponding quality certifications. The specifications of the quality certificate include: the purity, the absence of RNA and genomic DNA, the plasmid identity by sequencing and the plasmid homogeneity by densitometry in agarose gels.

These validation tools can be used for quality control laboratories of government, retailers, and fish industry. Actually, one of the objectives of this lecture is to set out the basic guidelines to set up a laboratory for fish identification by DNA analysis.