



# COMPARISON OF OLIGONUCLEOTIDE ARRAYS AND SPR-BIACORE SENSORS FOR FUNGAL DETECTION: TESTS OF ASPERGILLUS CARBONARIUS

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Fungi responsible for the presence of Ochratoxin A (OTA) in grapes have been identified as belonging to the black aspergilli, *Aspergillus* section *Nigri*, among which *Aspergillus carbonarius* is the main producer. OTA is a mycotoxin highly toxic for humans. Rapid methods for early detection of *A. carbonarius* in grapes are therefore required in order to plan appropriate control measures and protect consumer health.

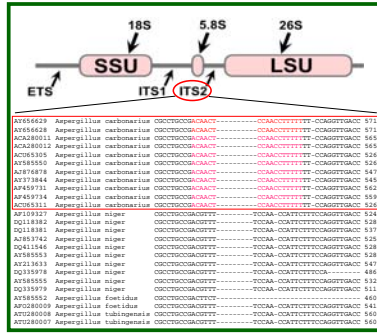
In order to rapidly identify the *A. carbonarius* species, specific sequences were selected in the ITS1 and ITS2 region of various *Aspergillus* species.

We tested two different methods to verify the specificity of various probes: macroarray method and SPR (Surface Plasmon Resonance) method.

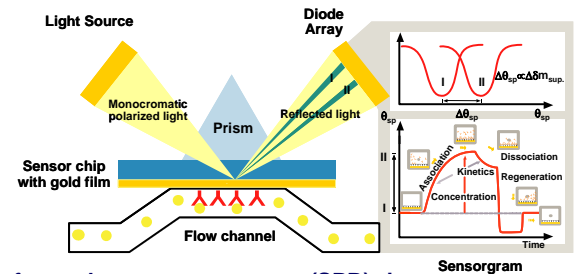
## DNA BASED SENSORS

Sequences of the ribosomal DNA (rDNA) from 337 strains belonging to 17 *Aspergillus* species were aligned and specific sequences were selected in the ITS1 and ITS2 region of various *Aspergillus* species.

Two generic primers (P1For, P2Rev) were also designed in order to amplify the fungal rDNA (including ITS1 and ITS 2 region).



## SURFACE PLASMON RESONANCE



Surface plasmon resonance (SPR) is a spectroscopic technique that measures refractive index changes occurring on a metal film, providing a signal that is positively correlated with the mass density changes of macromolecules on the sensing surface.

By immobilizing a biological recognition molecule (ligand) on the sensing surface, SPR biosensor provides a tool for the label-free analysis of biospecific interactions between the ligand and its binding partner (analyte) in solution.

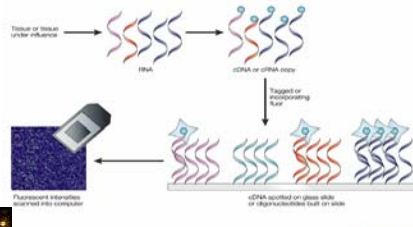
The refractive index change is measured in real time and plotted in a diagram (sensorgram) as resonance units (RUs) versus time, allowing both kinetic and analyte concentration analysis.

The biotinylated oligonucleotide, specific for *A. carbonarius*, was immobilized on a dextran-streptavidin pre-coated gold chip by means of the biotin-streptavidin interaction. The probe were tested for biospecific interactions with label-free amplified products obtained using DNA of *A. carbonarius*, *A. japonicus*, *A. aculeatus*, *A. ochraceus*, *A. niger*, *A. tubingensis* and *P. expansum*, and the primers P1For and P2Rev.

## DNA ARRAY FACILITY - BIOTECGEN

Molecular analysis by means of arrayed nucleic acids

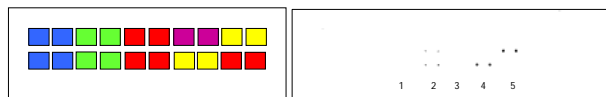
DNA array technology allows a high-throughput and simultaneous analysis of hundred of sequences



Nucleic acids labelling and hybridisation

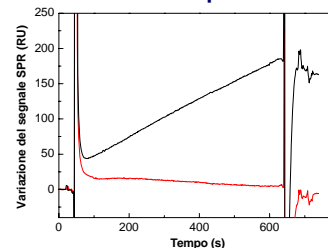
Signal Analysis of fluorescence intensity on a palette color scale

In the DNA macroarray experiments, oligonucleotides specific for *A. carbonarius*, *A. japonicus* and *A. ochraceus*, were provided with an NH2-terminal modification for covalent binding to epoxy-slides (Nexterion, Schott). These immobilised sequences were used as probe to test their specificity in hybridization experiments with the cyanine 3-labeled amplified products used as target. Fungal DNA was amplified with P1For and P2Rev generic primers.



- *Penicillium expansum*
- *Aspergillus carbonarius*
- *Aspergillus japonicus*
- *Aspergillus ochraceus*
- Universal oligonucleotide

A specific binding of the PCR product to the corresponding oligonucleotides (square green, n. 2) was obtained using the *Aspergillus carbonarius* DNA. The same probe binds to the Fungal-universal oligonucleotide (yellow). Species-specific oligonucleotides were spotted in quadruplicate.



Sensorgram showing the hybridization steps obtained only with the amplification product of the *A. carbonarius* DNA. No hybridization signal was obtained using as target the amplification products of the others *Aspergillus* and *Penicillium* species.

## CONCLUSIONS

We selected a sequence in ITS 2 region of the rDNA specific for *A. carbonarius*. The specificity was demonstrated by macroarray method and SPR- Biacore method. The advantage of the macroarray method consists in the possibility to immobilize a high number of different probes on the same chip, on the contrary, in SPR method, only two probes can be immobilized on the chip. Nevertheless the SPR method has the advantage that the amplified target DNA is label-free. The obtained results show that the two methods are both specific and sensitive.