

OLIGONUCLEOTIDE ARRAYS FOR DIFFERENTIATION OF *FUSARIUM* SPP BELONGING TO THE *LISEOLA* SECTION

Angela Quarta, Giovanni Mita, Palmiro Poltronieri, Antonio Logrieco, Angelo Visconti

Institute of Science of Food Productions, ISPA-CNR, via prov.le Lecce-Monteroni km 7, 73100 Lecce

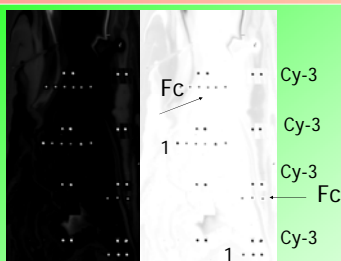


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INTRODUCTION

The *Liseola* section comprises several *Fusarium* species that are contaminant of many plants and plant products. Furthermore some of them can produce mycotoxins that are toxic for humans and domesticated animals. The exact knowledge of the species present as contaminants is thus important to predict the toxins that might be present in contaminated food or feed.

Fusarium verticilloides (*Gibberella moniliformis*) is a major contaminant of crops, one of the causing agents of maize-ear-rot and head blight in wheat; Diagnosis of contamination of toxin-producing *F. verticilloides* may help to prevent fumonisin B1 contamination; other species of interest in the *Liseola* section are *F. proliferatum* and *F. subglutinans* (Moniliformin, Beauvericin), *F. anthophilum* and *F. concentricum* (Beauvericin).



Fc: specific binding of the PCR product obtained using *F. concentricum* DNA to *F. concentricum* oligonucleotides (arrows).

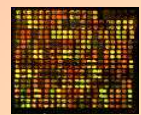
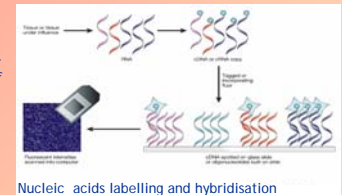
1: the same *F. concentricum*-probe binds to the *Liseola*-generic oligonucleotide.

Cy-3: positive controls, cyanine-3 labelled oligonucleotides spotted in duplicates. *Fusarium* species-specific oligonucleotides were spotted in triplicate.

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



Signal Analysis of fluorescence intensity on a palette color scale

THEMATICS: QUALITY OF FOOD PRODUCTS

Quality of agro-food products from plants



Quality of agro-food products of animal origin



RESULTS

EF-1 α elongation factor partial sequences of 28 species of *Fusarium* in the section *Liseola*, available in the ITEM collection (www.ispa.cnr.it/Collections) were aligned by ClustalW. The alignment of the various sequences revealed differences that allowed the selection of different primers potentially able to differentiate each species. 20-mer oligonucleotides were designed and made synthesized having an NH₂-terminal for covalent binding to epoxy-slides (Nexterion, Schott).

Generic primers for the PCR amplification of the *Fusarium* elongation factor 1- α were also selected and the amplified products were cyanine 3- labeled during the PCR reaction, and purified by gel-filtration.

The PCR probes were added to the hybridisation buffer and incubated with the oligonucleotides bound on the array.

Hybridisation experiments were carried out and the results (as shown in the figure) show that all the designed oligonucleotides are specific for each species.