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Oligonucleotide Microarray for the Identification of Potential Mycotoxigenic Fungi on Crops

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INTRODUCTION

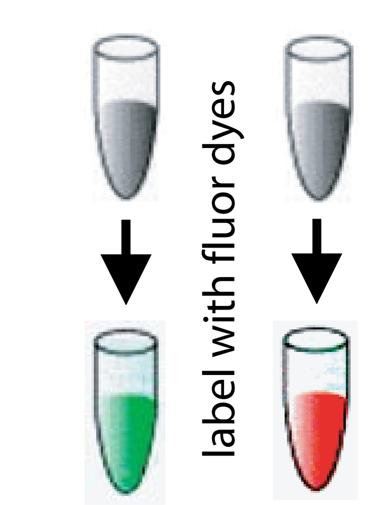
Mycotoxins are fungal toxins which pose a continuous challenge to the safety and quality of food commodities in South Africa. These toxins have been associated with unthriftiness, loss of appetite, feed refusal, liver cirrhosis, vomiting, poor weight gain, various cancers and mortality on humans and animals that eat contaminated foods. In this study a diagnostic microarray was developed to identify 40 potentially mycotoxigenic fungi as well as genes leading to toxin production in both laboratory and food samples. For fungal discrimination, the polymorphisms of the internal transcribed spacer (ITS) regions and the elongation factor 1- alpha (EF-1 α) gene were exploited for the design of the oligonucleotide probes. For some probes with similar sequences, the specificity of a probe was increased in some instances by substituting an oligonucleotide with a high affinity DNA analogue known as locked nucleic acid (LNA). For the detection of fungi that can produce mycotoxins, oligonucleotides for the genes leading to mycotoxin production were selected from public databases and included in the oligonucleotide array. The oligonucleotides selected for fungal identification and the oligonucleotides specific for toxin producing genes were spotted onto microarray slides.

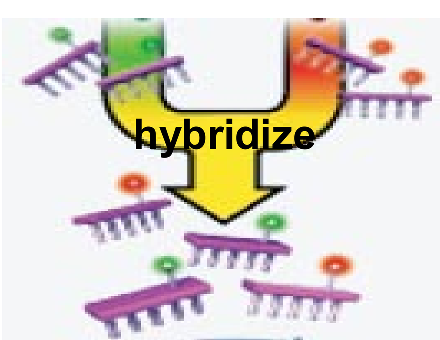
METHODS

The complete microarray procedure

Extract genomic DNA

Reference Test





Step 1-3: Fungal and control DNA are labeled with fluorescent dyes

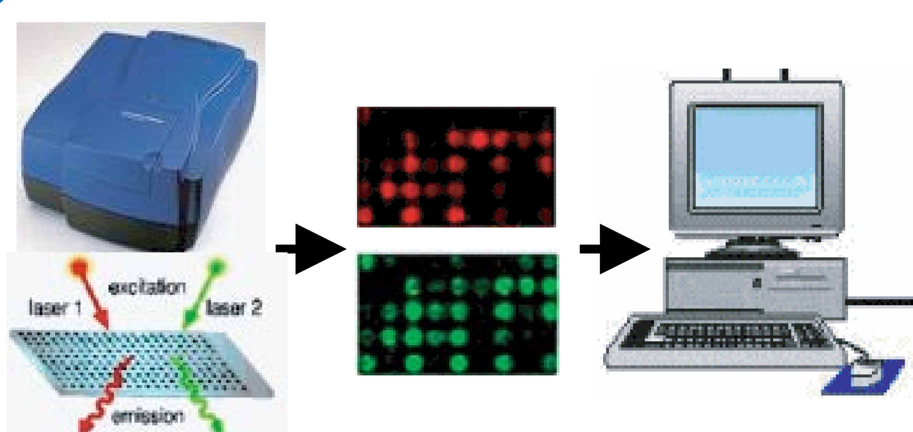
Step 4: Fungal and control DNA are applied to the microarray and compete to attach or hybridize to the microarray

Step 5: The microarray scanner measures the fluorescent signals

Step 6: Computer software analyses the data and binary scores are obtained

Print slides

Design oligonucleotides



Scan

Computer analysis

RESULTS

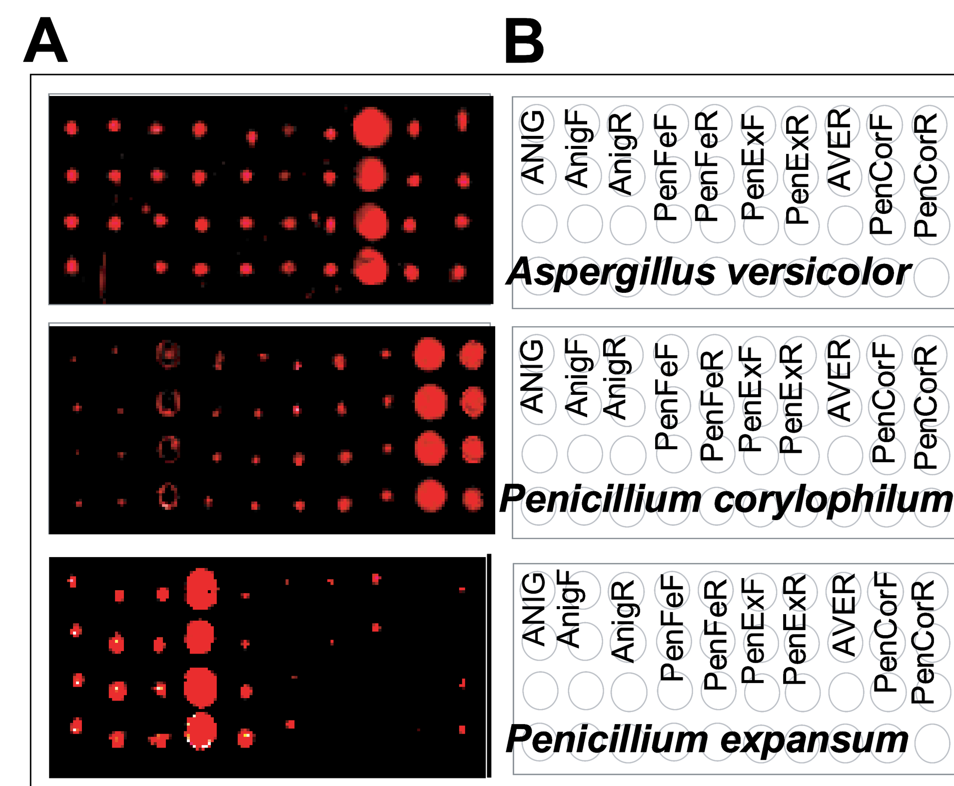


Figure 1: A. Hybridization profile of *Aspergillus versicolor* (Top); *Penicillium corylophilum* (Middle); *Penicillium. expansum* (Bottom). B. The arrangement of a few oligonucleotide probes within the indicated fields of a section of the array. Each column represents four replicates of the same spot.

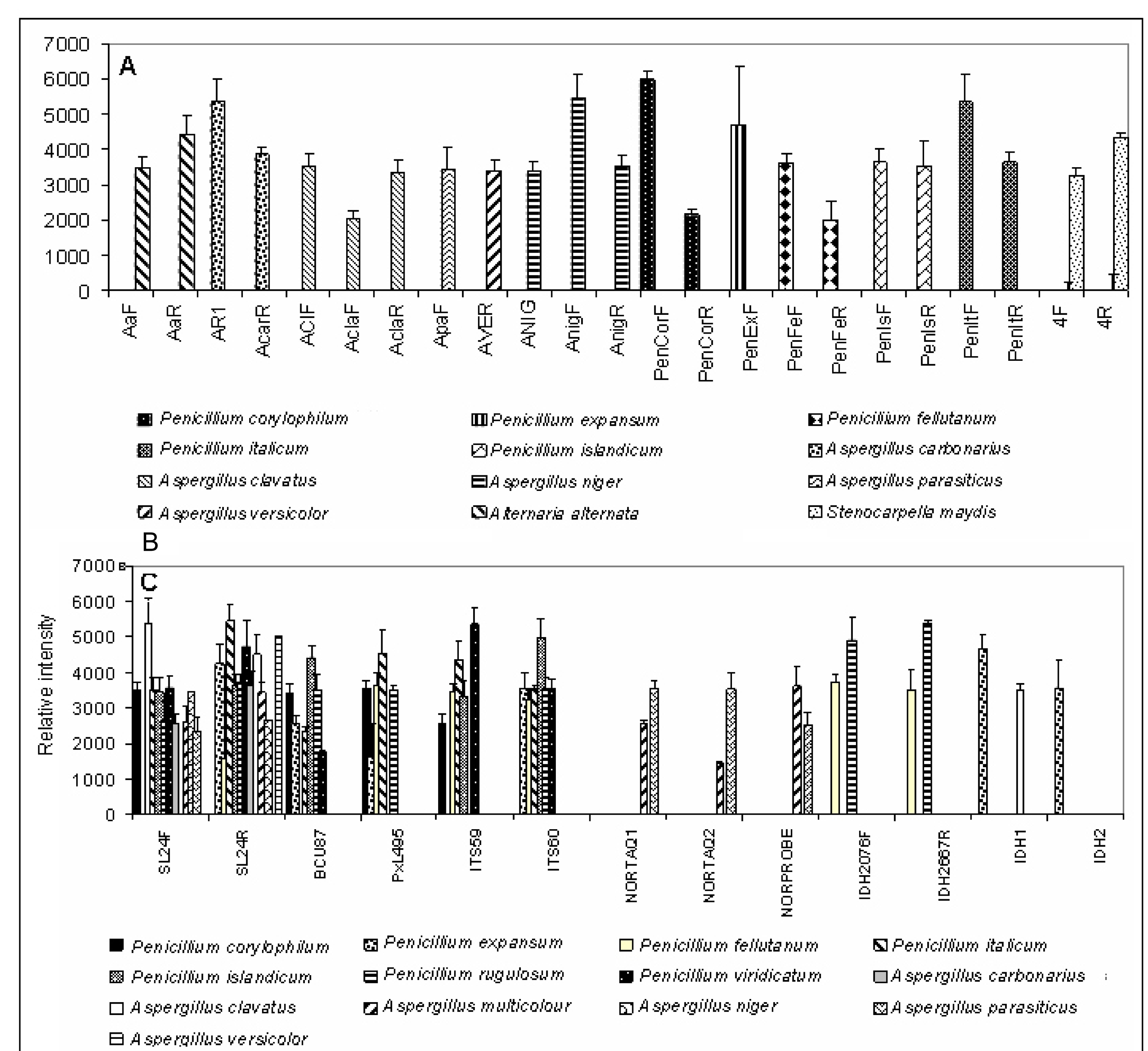


Figure 2: Relative intensities after hybridization of labeled target DNA to the array. Only positive hybridization results are shown. A. Relative intensities of fungal strains hybridizing to probes designed from the internal transcribed (ITS) regions of *Alternaria, Aspergillus, Penicillium* and *Stenocarpella* species. B. Relative intensities of Alternaria, Aspergillus, Penicillium and Stenocarpella species hybridizing to their relevant mycotoxin genes.

DISCUSSION AND CONCLUSIONS

Oligonucleotide microarrays have a high multiplexing capacity and have been shown to be efficient for the analysis of many samples simultaneously. This technology offers an accurate identification process based on sequence confirmation through hybridization [Vora *et al.*, 2004] and has the ability to detect more than one parameter at a time, in this case fungal species and genes involved in pathways leading to toxin production. A total of 32 fungi were characterized and their potential to produce mycotoxins could be identified. For the eight species that could not be identified to species-level, additional probes need to be designed to discriminate between these species. The presence of genes leading to toxin production could also be determined, but it was not possible to predict whether the mycotoxin was expressed. In future studies, the developed microarray chip can be used to hybridize DNA and cDNA labeled with different Cy dyes to the array. The DNA can determine the fungal identity and the cDNA can determine whether genes for mycotoxin biosynthesis are expressed in a crop plant.

REFERENCES

[1] Vora GJ, Meador CE, Stenger DA, Andreadis JD: Nucleic acid amplification strategies for DNA-microarray-based pathogen detection. *Appl Environ Microbiol* 2004, **70**:3047-3054