Dynamics and Biodiversity of microorganisms (fungi, yeast and bacteria) by PCR-DGGE, influencing OTA production on coffee beans

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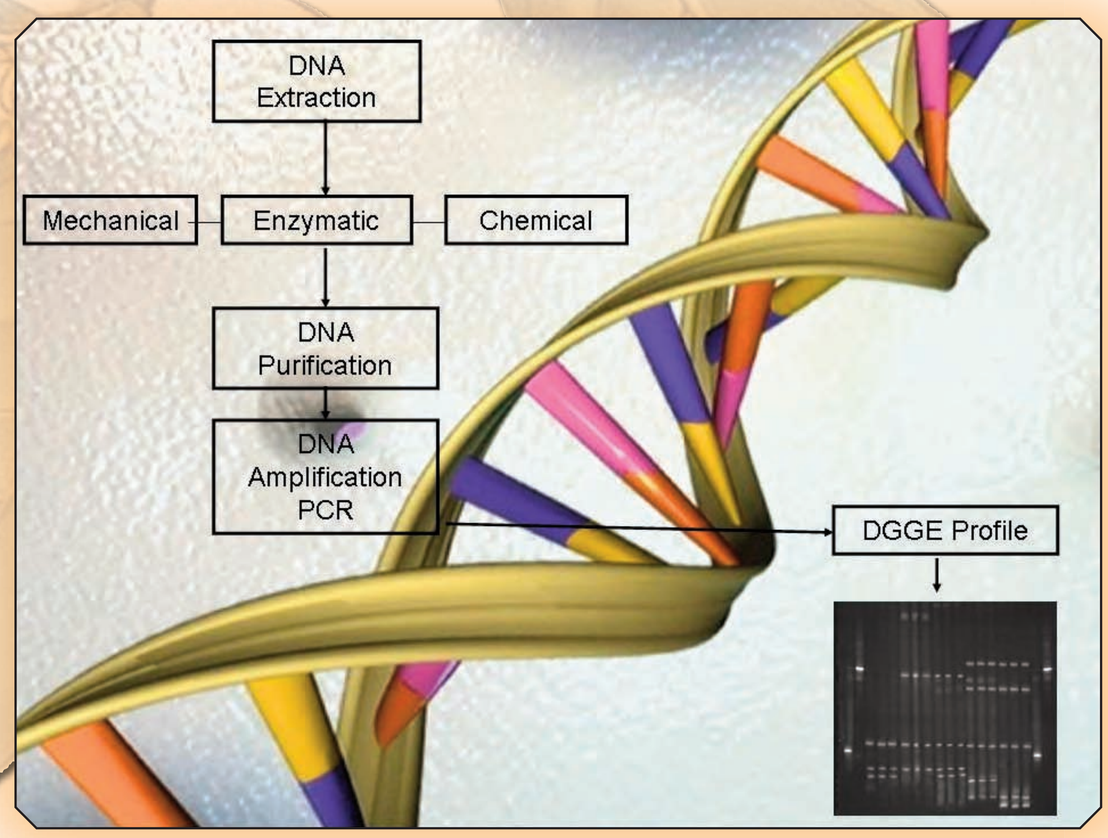
Ochratoxin A is a secondary metabolite produced by various filamentous fungi, is deemed to have nephrotoxic, immunotoxic, teratogenic and carcinogenic effects (1). In tropical zones, OTA is mainly produced in coffee beans by three *Aspergillus* species: *A. carbonarius, A. niger* section Nigri and *A. ochraceus* section Circumdati. In temperate zones *Penicillium verrucosum* and *P. nordicum* are known to synthesize OTA in food commodities (2, 3).

The OTA content in coffee was shown to be closely link to harvesting conditions, post-harvest processing conditions and especially dry processing, storage and transportation conditions (4, 5, 6). In order to understand the OTA contamination process in foodstuffs, assays on PCR-DGGE (Polymerase Chain Reaction - Denaturing Gradient Gel Electrophoresis) were carried out. PCR-DGGE is a rapid molecular technique that was developed to monitor the dynamics of microbial populations (fungi, yeast and bacteria) per example in coffee mycoflora. In this work, we optimized PCR-DGGE stages: extraction and amplification, repeatability and sensibility of the fungi methodology were tested.

Methodology

• **Sensibility** test was applied to selected Aspergillus species cultivated on Potato Dextrose Agar medium (AES, Combourg, France) for 5 days at 25°C. The fungal spores were collected from plates in aseptic conditions using sterile distilled water with 0.1% Tween 80 solution.
The suspensions of fungal spores were quantified with Thomas cell and dilute in peptone water from 10^7 to 10^2 spores/mL.

• **DNA extraction method,** according to El Sheikha *et al.* (7) different steps of extraction were used (mechanical/enzymatic/chemical). Additionally, the successful application of a eukaryotic universal primer for PCR permitted to amplify and identify many fungi species in one PCR step. The PCR products were analyzed by DGGE by using a Bio-Rad DcodeTM universal mutation detection system (Bio-Rad Laboratories, USA) using the procedure described by El Sheikha *et al.* (8).



Results

• Sensibility

Sensibility was examined on pure cultures of 3 fungal species at different concentrations: *Aspergillus ochraceus, A. carbonarius, A. niger.* Extractions were performed directly on spores. Results were considered as positive when PCR amplification was obtained. The detection limits in pure cultures are shown in Table 1.

Table 1: Detection limits on pure cultures of fungal strains

Fungi/Concentration (spores/mL)	10^7	10^6	10^5	10^4	10^3	10^2
Aspergillus carbonarius	+	+	+	+	-	-
Aspergillus niger	+	+	+	+	-	-
Aspergillus ochraceus	+	+	+	+	-	-

• Repeatability

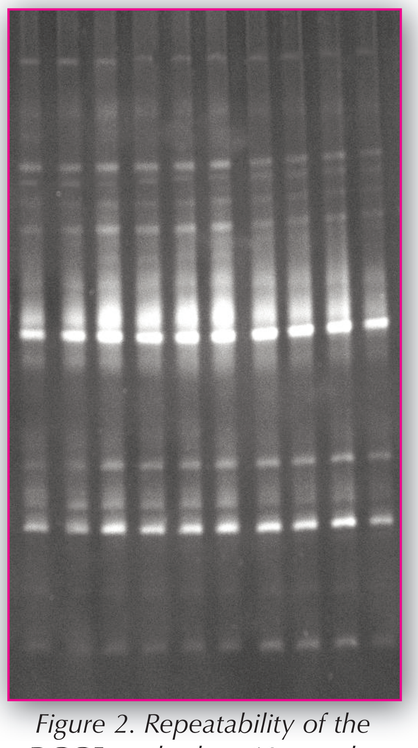


Figure 2. Repeatability of the DGGE method on 10 samples from the same batch.

• Profil DGGE

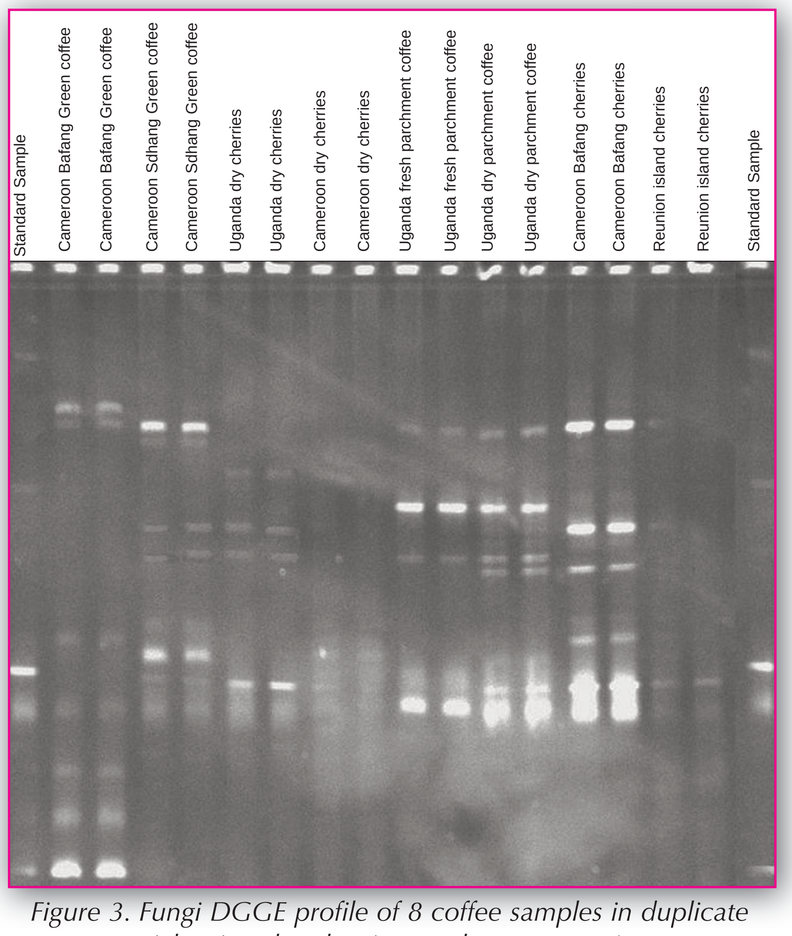


Figure 3. Fungi DGGE profile of 8 coffee samples in duplicate (cherries, dry cherries,parchment, green).

Conclusion

THE sensitivity and the repeatability of the protocol were increased with the improvement of the DNA extraction and the PCR/DGGE conditions (Table 1, Fig. 2).

The dynamics of fungus populations linked to OTA production, as well as post-harvest phytopathogens, could be studied by fingerprinting with PCR-DGGE (Fig. 3). The advantages of this method are its efficiency on all microbial species (fungi, yeast and bacteria) and on the possibility of analysing a wide number of samples (30 samples) in a unique batch. PCR-DGGE provides a method for tracing microorganisms among the technological treatments (Fig. 3).

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