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

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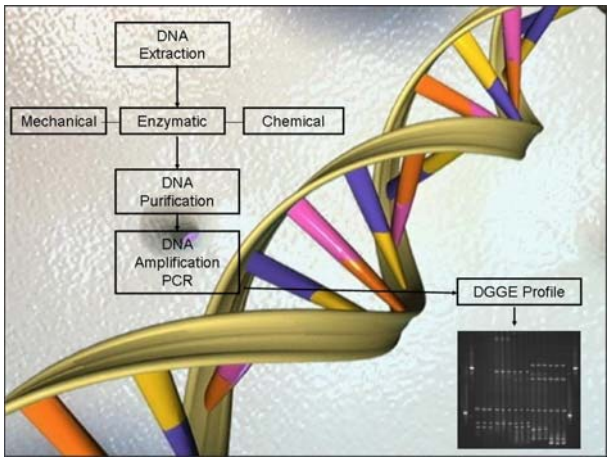
Ochratoxin A is a secondary metabolite produced by various filamentous fungi contaminating in a wide range of food and animal feedstuffs. OTA has been shown to possess nephrotoxic, carcinogenic, immunodepressive and teratogenic properties (1).
In tropical zones, OTA is mainly produced in coffee beans by three Aspergillus species: *A. carbonarius*, *A. niger* section Nigri and *A. ochraceus* section Circumdati. Among them, the most important OTA producer and the most frequently isolated is *A. carbonarius*. In temperate zones *Penicillium verrucosum* and *P. nordicum* are known to synthetize 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 some producing countries, damaged caused on beans by other fungal communities undoubtedly lead to high OTA contents in coffee.
In order to understand the OTA contamination process in foodstuffs, PCR-DGGE (Polymerase Chain Reaction - Denaturing Gradient Gel Electrophoresis) assays were carried out on coffee microflora. PCR-DGGE is a rapid molecular technique that was developed to monitor the dynamics of microbial populations (fungi, yeast and bacteria). PCR-DGGE is used to characterize the microbial flora of food products by extraction and amplification of 16S, 26S and 28S rDNA for bacteria, yeast and fungi. PCR-DGGE stages i.e: extraction and amplification were optimized. Detection limits are estimated on several fungus, yeast, and bacteria. Additionally, repeatability and sensibility of the methodology were also tested. In this study only fungi **methodology** are showed.

Methodology
We applied the sensibility test on Aspergillus species as one of fungal genera which selected and cultivated on potato dextrose agar **(PDA) as cultural medium** (AES, Combourg, France) for 5 days at **25°C**. Fungal spores were harvested from plates in aseptic conditions using sterile distilled water with 0.1% Twee**n** 80. The suspensions of fungal spores were quantified with Thomas cell and dilute in **peptone** water.
The purpose of this study was to apply the extraction method which combined mechanical/enzymatic/chemical according to El Sheikha *et al.* 2009**a** (7). 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 first described by **El Sheikha *et al.* 2009b** (**8**) **F**ig 1.



Results

- Sensibility

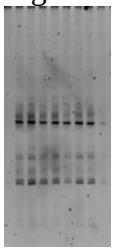
Sensitivity was examined on pure cultures of 3 fungal species at different concentrations: *Aspergillus ochraceus*, *A. carbonarius*, *A. niger*. Extractions were performed either directly on spores Results were considered positive when we obtained a PCR amplification even if very **small** quantity of DNA was extracted and **invisible** on the **0.8%** agarose gel. The detection limits in pure cultures are shown in Table 1.

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

Table 1: Detection limits on pure cultures of fungal strains

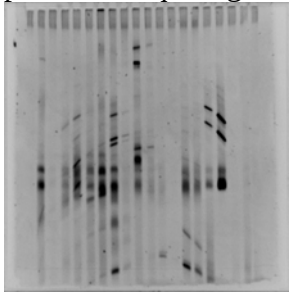
- Repeatability.

Fig 2: Repeatability of the method DGGE on 9 samples of the same batch.



- Profil DGGE

Fig 3: **Example** of DGGE **profile of** fungi on 8 samples in duplicate of coffee (berry, parched, coques, green).



Conclusion

The dynamics of fungus populations linked to OTA production as well as post-harvest phytopathogens could be studied by PCR-DGGE genetic fingerprinting. The advantages of this method are the efficiency on all microbial species (fungi, yeast and bacteria) and the possibility of analysing a wide number of samples (30 samples) in a unique step.

It is seen that PCR-DGGE seems to provide a method not only for tracing the geographic origin but also the technological treatments (Fig. 3). The extraction protocol allows to increase the sensitivity by 4 log in three fungal species. We confirmed the results by repeatability experiment which showed the same profile (Fig. 2).

References

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