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# Determination of coffee origin by using 28S rDNA fingerprinting of fungal communities by PCR-DGGE: Application to the Cameroonian coffee

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**Abstract**

The new European regulation 178/2002 imposes the determination of the geographical origin in the traceability process of foodstuffs at the moment of commercial transactions. In practice, it is difficult to determine with accuracy the geographical origin of foodstuffs. For this purpose, the total analysis of fungal communities in samples of coffee is used. In the present study the molecular technique using 28S rDNA profiles generated by PCR-DGGE was used in order to detect the variation in fungal community structures of coffee from five different locations in West and Coastal plain in Cameroon and the effect of treatment and coffee species on these fungal profiles. When the 28S rDNA profiles were analyzed by multivariate analysis, distinct microbial communities were detected. The band profiles obtained from different samples of coffee and specific for each location and could be used as a bar code to certify the origin of the coffee. This method is a new traceability tool which provides coffee products with a unique biological bar code and makes it possible to trace back the coffee to their original location.

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**Introduction**

International trade intensifies and extends to the entire planet. Foodstuffs are often consumed far from their zone of production. The consumer is more and more demanding and sensitive to the quality and the origin of the foodstuffs that they buy. Issues surrounding food safety and security continue to be hot topics that concern the whole the supply chain. Food crises such as bovine spongiform encephalopathy and avian influenza remain embedded in the memories of European customers. Regulations across Europe continue to be tightened in order to provide a greater degree of assurance in quality and safety. Meanwhile, the traceability and labeling of imported products in European countries remains a compulsory issue (UE Regulation No. 178/2002).

Coffee is produced by processing fruits of the genus *Coffea*. Two species dominate the world market, *Coffea arabica* (yielding coffee variety *arabica*) and *Coffea canephora* (yielding coffee variety *robusta*). Coffee production in Cameroon is shared between producers, grouped into farmer cooperative societies (FCS) and estates (E). FCS farmers practice extensive cultivation of few acres of coffee trees with very few or no inputs. Conversely, estates practice intensive coffee cultivation, where coffee plants are kept in good phytosanitary condition by regular applications of fungicides and insecticides (FAO, 2006). Cameroonian producers retain ownership of their coffee up to its sale to exporters or roasters. The price paid for coffee depends on its quality when it goes up for auction. As a consequence, this calls for perfect traceability of coffee from the production unit to the warehouses in which the coffee entering the market is stored. Microflora of coffee beans is composed of yeasts, bacteria and fungi, which could have deep impacts on coffee quality (Sylva *et al*., 2000). Undesirable micro-organisms present on coffee beans before and/or during transformation can irremediably cause detrimental, sensorial or chemical defects. Fungi are responsible for coffee diseases (mildew and black rot), mycotoxin production (Cabanes *et al*., 2002; Battilani *et al*., 2003) or sensorial defects in coffee such as musty or earthy aromas (La Guerche *et al*., 2004). Knowledge of the structure and diversity of the fungal community of coffee beans would lead to a better understanding of the emergence of defects in coffee in relation to fungi presence on coffee beans.

For economic reasons and for profitability, several batches of coffee of various origin or various cultivars could be mixed. It is thus very difficult to check their exact geographical origin. Traceability is only assured by rigorous labeling and administrative documentation however it is not under control. In case of doubt or fraud, it is necessary to find a precise and rapid analytical technique in order to determine the geographical origin. The most popular analytical methods that allow us to ensure the determination of origin are bar coding, spec-troscopy, stable isotopes, etc. (Peres *et al*., 2007). Stable isotopes are the only methods that are referenced as an European regulation for wine origin determination (Ghidini *et al*., 2006). Thus, it seems difficult to use coffee genomic markers to ensure the traceability. However, the skin of coffee is not sterile and can carry microorganisms or their fragments. The presence of various microorganisms must depend on the external environment of the coffee (soil ecology, spoilage, insects, diseases), but also microorganisms brought by human activity (Sodeko *et al*., 1987).

The idea was to create a “biological bar code” (Montet *et al*., 2004), based on the analysis of the DNA of microorganisms present on the products. This method is based on the assumption that the microbial communities of the coffee are specific for a geographical area (Le Nguyen *et al*., 2008; Montet *et al*., 2008).

The main objective of this study was to apply the PCR–DGGE method to analyze in a unique step all the moulds present on the coffee, in order to create an

analytical technique that will permit the linkage of fungal communities to the geographical origin and avoid the individual analysis of each moulds strain. The acquired band patterns for the fungal communities of different species of coffee and different harvesting locations were compared and analyzed statistically to determine the coffee geographical origin. To the best of our knowledge, this paper describing a molecular method, PCR–DGGE that will permit the certification of the coffee origin, in particular by using 28S rDNA fingerprinting of moulds.

## Materials and methods

*Coffee samples*

Before sample collection investigations were made on the field with farmers every three months for one year. Fig. 1 recapitulates this field work with samples collected at stages $I_a$, $I_b$ and II of the process. Samples of 500g of coffee (parchment, coffee cherries and coffee beans) (stage $I_a$, $I_b$, II) were collected from five different sites of the west regions and the coastal plain in Cameroon, during the campaigns of 2009 and 2010. The samples were then placed in sterile plastic bags and sent for laboratory analysis.

*Fungi analysis*

Subsamples of parchment coffee, dry cherry or green coffee were also plated directly onto Petri dishes (five beans per plate) containing Dichloran 18% Glycerol agar (DG18) (Hocking and Pitt, 1980; Guiraud, 1998) and Potato dextrose Agar (PDA) without prior superficial disinfection. Plates were incubated at 25°C for 5 to 7 days; results were expressed as a rate of contaminated coffee beans. Moulds were isolated, sub-cultured and observed with an optical microscope for preliminary identification. This was done by morphotypic analysis of the colony, especially color and appearance using the proposals of Pitt and Hocking (1997). Only genera of *Penicillium, Fusarium* and *Aspergillus* were further identified to species level.

*Fungal DNA isolation and PCR amplification*

Fungal spores were harvested from plates in aseptic conditions using sterile distilled water with 0.1% Tween 80. Extraction method which combined mechanical/enzymatic/chemical was applied according to El Sheikha *et al.* (2009).

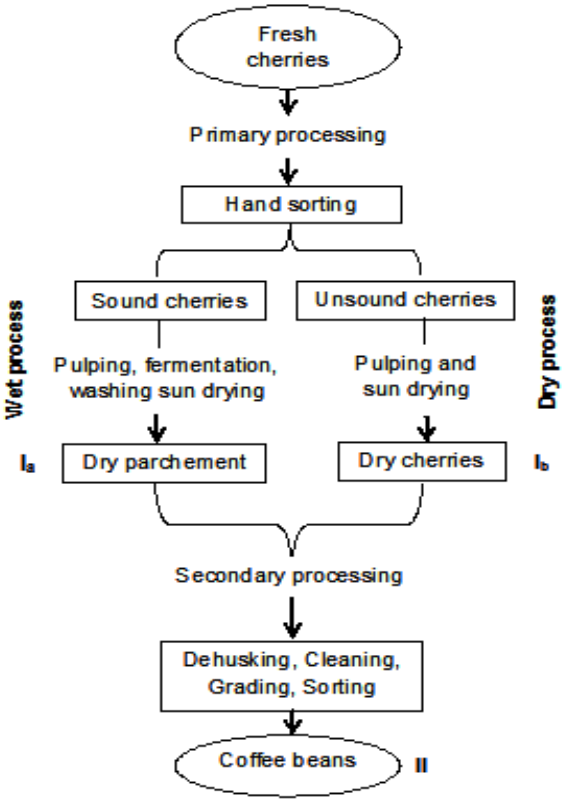


**Fig. 1.** Coffee processing steps from fresh cherries to coffee beans in Cameroon. $I_a$, $I_b$ II: sampling steps.

DNA was quantified spectrophotometrically with a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, USA) and kept at – 20°C until used as template for PCR amplification. Additionally, the successful application of a eukaryotic universal primer for PCR permitted to amplify and identify many fungi species in a unique PCR step.

A fragment of the D1/D2 region of the 28S rDNA gene was amplified using eukaryotic universal primers: forward, U1f GC (5' - CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GTG AAA TTG TTG AAA GGG AA – 3'; Sigma); reverse, U2r (5' - GAC TCC TTG GTC CGT GTT – 3'; Sigma), amplifying a 260 bp fragment

(Möhlenhoff *et al*., 2001; Khot *et al*., 2009; El Sheikha and Montet, 2010; El Sheikha *et al*., 2010). A 30 bp GC-clamp (Sigma) was added to the forward primer PCR was performed in a final volume of 50 µL containing 0.2 µM of each primer, all the deoxyribonucleotide triphosphate (dNTPs) at 200 µM, 1.5 mM of $MgCl_2$, 5 µL of $MgCl_2$-free 10xTaq reaction buffer (Promega), 1.25 Units of Taq DNA polymerase (Promega) and 2 µL of extracted DNA (≈ 30 ng). The amplification was carried out as follows: An initial denaturation at 94°C for 3 min, 30 cycles of 94°C for 45 sec, 50°C for 50 sec, 72°C for 90 sec and a final extension at 72°C for 5 min.

PCR products were verified by electrophoresis by loading 5 µL on 2% TAE agarose gels with a 100 pb molecular weight ladder. Gels were stained and photographed as mentioned above.

*DNA extraction from coffee moulds*

8 grams of each coffee beans sample were taken aseptically and filled into sterile 50 mL tubes containing 10 mL of sterile peptone water. The tubes were placed on a rotating wheel for 1 h. 2 mL of supernatant were then collected into Eppendorf tubes of 2 mL containing 0.3 g of glass beads. Extraction was done following the protocol of El Sheikha *et al*. (2010). DNA extraction samples were verified by electrophoresis and loaded into 0.8% agarose gels in 1 × TAE buffer (40 mM Tris–HCl, pH 7.4, 20 mM sodium acetate, 1.0 mM $Na_2$–EDTA; Eppendorf, Germany) with a molecular weight ladder (Supercoiled DNA Ladder 16.21 kb; Invitrogen, USA). After running at 100 V for 30 min, the gels were stained for 30 min in an ethidium bromide solution (50 µg/mL; Promega), rinsed for 20 min in distilled water, then observed and photographed on a UV transilluminator, using a black and white camera (Scion Co., USA) and Gel Smart 7.3 system software (Clara Vision, Les Ulys, France).

*Denaturing Gradient Gel Electrophoresis (DGGE) analysis*

The PCR products were analyzed by DGGE, by using a Bio-Rad Dcode universal mutation detection system (Bio-Rad, USA), using the procedure first described by Muyzer *et al*. (1993) and improved by Leesing (2005). Samples containing approximately equal amounts of PCR amplicons were loaded into 8% w/v polyacrylamide gels (acrylamide: N,N'-methylene bisacrylamide, 37.5 : 1; Promega) in 1 × TAE buffer (40 mM Tris–HCl, pH 7.4, 20 mM sodium acetate, 1.0 mM $Na_2$-EDTA).

All electrophoresis experiments were performed at 60°C, using a denaturing gradient in the 40–70% range (100% corresponded to 7 M urea and 40% v/v of formamide; Promega). The gels were electrophoresed at 20 V for 10 min and then at 80 V for 16 h. After electrophoresis, the gels were stained for 30 min with ethidium bromide and rinsed for 20 min in distilled water and then photographed on a UV transilluminator with the Gel Smart 7.3 system (Clara Vision, Les Ulys, France).

*Identification of DGGE bands*

Detected bands were cut from the DGGE gel with a sterile scalpel. DNA of each band was then eluted in 100 µL TE buffer at 4°C overnight. 100µL of DNA eluted from each band was purified and sent for sequencing as described above (in section Fungal DNA isolation and PCR amplification) but using U1 primer with no GC clamp, and then sent for sequencing at GATC Biotech (Germany).

Sequences were finally compared to those in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) and those of the Ribosomal Database Project (http://rdp.cme.msu.edu/index.jsp) using the BLAST program (Altschul *et al*., 1997). Sequences with a percentage identity of 97% or greater were considered to belong to the same species (Stackebrandt and Goebel, 1994; Palys *et al*., 1997).

*Image and statistical analysis*

Individual lanes of the gel images were straightened and aligned using Image Quant TL software v. 2003 (Amersham Biosciences, USA). Banding patterns were standardized with three reference patterns included in all gels, Wickerhamomyces anomalus DNA and Komagataella pastoris DNA. This software permitted identification of the bands relative positions compared with standard patterns. In DGGE analysis, the generated banding pattern is considered as an image of all of the major yeast in the populations. An individual discrete band refers to a unique 'sequence type' or phylotype (Van Hannen *et al.*, 1999; Muyzer *et al.*, 1995). This was confirmed by Kowalchuk *et al.* (1997), who showed that co-migrating bands generally corresponded to identical sequences. The DGGE fingerprints were manually scored by the presence and absence of co-migrating bands, independent of intensity. Pairwise community similarities were quantified using the Dice similarity coefficient (SD) (Heyndrickx *et al.*, 1996): $S_D = 2N_c/N_a + N_b$

Where $N_a$ represents the number of bands detected in sample A, $N_b$ the number of bands in sample B, and $N_c$ the number of bands common to both samples. The similarity index was expressed within a range of 0 (completely dissimilar) to 100 (perfect similarity). Dendograms were constructed using the stat graphic plus version 5.1 software (sigma plus, France). Similarities in community structure were determined using the cluster analysis with Euclidian distance measure.

**Table 1.** Comparison of dominant moulds species identified during technological treatments of coffee by classical culturing techniques and by DGGE.

| Sample[a] | Species identified by culturing | Species identified by DGGE |
|---|---|---|
| **Wet process: stage $I_a$** | | |
| **3409** | *A. carbonarius; A. niger; P. roqueforti* | *A. nomius ; A. fumigatus ; P. citrinum ; Wallemia muriae ; W. sebi ; roqueforti; Mucor sp.* |
| **3709** | *A. niger ; P. roqueforti; Fusarium lacertarum* | *A. niger ; P. citrinum ; W. sebi* |
| **3410** | *A. carbonarius; A. niger; P. citrinum* | *A. carbonarius; A. niger; A.ochraceus; Acremonium murorum; P. roqueforti; W. muriae; P. citrinum; P. arenicola* |
| **4210** | *A. carbonarius; A. niger; F. lacertarum,* | *Mucor sp.; W. sebi; P. roqueforti; Acremonium murorum* |
| **Dry process: stage $I_b$** | | |
| **1209** | *A. carbonarius; Rhizopus nigricans ; P. roqueforti* | *A.niger; A.carbonarius; Acremonium murorum; W. muriae* |
| **0510** | *A. niger; R. nigricans ; P. roqueforti ; F. chlamidosporum* | *A.niger; A. carbonarius; A. ochraceus; W.sebi; P. citrinum ; P. arenicola, P. roqueforti* |
| **0610** | *A. niger; R. nigricans ; P. roqueforti ; F. chlamidosporum* | // |
| **1210** | *A. niger ; F. lacertarum* | *A.niger, A.carbonarius; P. citrinum ; P. arenicola, P. roqueforti* |
| **1710** | *A. carbonarius; A. niger; R. nigricans ; P. roqueforti* | *A.niger; A. nomius ; W. sebi ; Acremonium murorum; W. muriae* |

| | | |
|---|---|---|
| **2510** | *A. carbonarius; A. niger; P. roqueforti ; A. tamarii* | *A. nomius ; w. sebi ; P. roqueforti; Mucor sp.* |
| **3810** | *A. niger; R. nigricans ; P. citrinum* | // |
| **1110** | *A. carbonarius; R. nigricans ; Mucor hiemalis* | *A. carbonarius; A. ochraceus; W. muriae; P. citrinum; P. arenicola* |
| | **Dry and Wet process: stage II** | |
| **0410** | *A. niger; R. nigricans ; M. hiemalis ; A. flavus* | *A. niger; A.carbonarius; W. sebi; P. citrinum; P. arenicola* |
| **2710** | *A. carbonarius; R. nigricans* | *A. nomius; P. roqueforti; W.sebi; Acremonium murorum; Mucor sp.* |
| **3910** | *A. niger; F. lacertarum ; mucor sp.* | *A.nomius; A.fumigatus; A. carbonarius; A. ochraceus; W. sebi; W. muriae; Mucor sp.* |
| **4010** | *A. carbonarius; A. niger; A. tamarii* | *A.nomius; W. sebi; P. citrinum; P. roqueforti; Acremonium murorum; Mucor sp.* |

[a]Sample number assigned for laboratory control; Ia, Ib, II: stage of process

**Table 2.** Characteristics of samples coffee used for the comparison of fungal profiles obtained by PCR-DGGE

| Reference | | 3410 | 0610 | 1409 | 41/9 | 3109 | 2710 | 2510 | 0909 | 3110 | 4810 | 5110 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Type | Marker (M) | P | V | P | V | V | V | C | V | C | V | C |
| Variety | | A | R | A | R | R | R | R | R | R | R | R |
| Treatment | | VH | VS | VH | VS | VS | VS | VS | VS | VS | VS | VS |
| | | VHD | VSD | VHB | VS1B | VSB | VSS | VS1S | VSA | VS1A | VSM | VS1M |
| locations | | DSH | | | BFSS | | SAN | | BAF | | MEL | |

P : parchement ; V : Green coffee ; C :Dry cherries ; A : arabica ; R : robusta ; VH : wet process ; VS : Dry process ; DSH : Dscang ; BFSS : Bafoussam ; SAN : Santchou ; BAF : Bafang ; MEL : Melong

## Results

*Extraction of fungal DNA and verification of PCR amplification of the extracted DNA*

DNA extraction of the fungal community was performed on coffee, with and without husks. Extraction efficiency was verified with a 0.8% w/v agarose gel. The DNA extraction method showed high efficiency and bands with a molecular weight >16 kb corresponding to genomic fungi DNA were clearly observed on the gel.

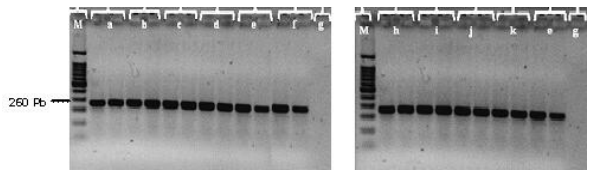


**Fig. 2.** Banding pattern of PCR reactions of isolated filamentous fungi (M: marker 100pb; g: negative reference; a – e: Moulds DNA).

Fungal DNA obtained after extraction was amplified by classical PCR, adapted from Kurtzman and Robnett (1998) and Cocolin *et al.* (2000). PCR amplicons were analyzed by electrophoresis on 1% w/v low melting agarose gel at 100 V for 30 min in TEA buffer, as described above. All bands were clearly observed and had a molecular weight of the expected size of the amplicon (Fig. 2). The high intensity of the bands representing the PCR amplicons indicated that PCR amplification yielded high quantities of fungal DNA allowing further analysis by DGGE (from coffee samples) and direct sequencing (of pure strains isolated from coffee) to be completed.

*Mycological analysis and identification of fungal isolates by sequencing*

As part of this work, analysis of the fungal flora was made on coffee samples taken at different stages of coffee processing method as shown in Fig. 1. Of these samples, the fungal flora was characterized by traditional techniques and by sequencing bands obtained by PCR-DGGE.

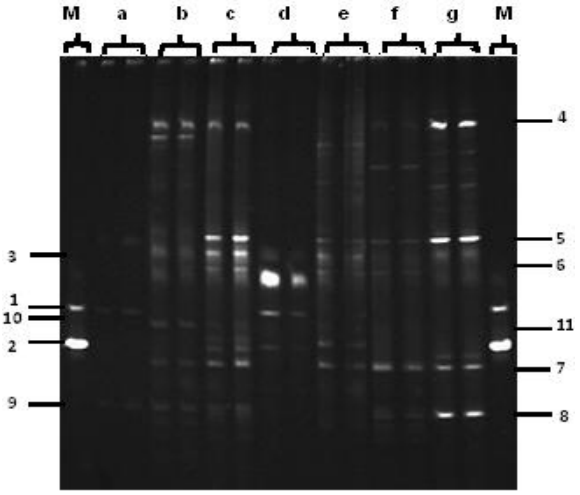


**Fig. 3.** DGGE profiles of PCR amplicons of the domain D1 of 28S rDNA that represent the fungi biodiversity in samples taken during treatment of coffee (M: marker; a-g: samples of coffee (3709, 3409, 3910, 1209, 1710, 2710, and 2510). The position of bands is indicated by numbers that correspond to species of fungi: 1*: A. niger;* 2: *A. carbonarius;* 3: *Wallemia muriae;* 4: *Mucor sp.;* 5: *Wallemia sebi*; 6: *Acremonium murorum;* 7 *A.nomius;* 8: *Penicillium roqueforti;* 9: *P. citrinum;* 10: *A. fumigatus;* 11: *A. ochraceus.*

Table 1 shows the comparison of dominant moulds species identified during technological treatments of coffee by the classical culturing techniques and by DGGE. The use of direct plating of beans in Petri dishes on DG18 agar and PDA for samples (Table 1) was employed. A high level of infection by moulds was observed on parchment and dry cherries in all processes after drying. Eleven different species have been identified by conventional techniques, they were: *Aspergillus niger, A. carbonarius, A. flavus, A. tamarii, Fusarium chlamidosporum, F. lacertarum, Mucor hiemalis, Mucor sp., Penicillium citrinum, P. roqueforti, Rhizopus nigricans.*

*DGGE analysis of representative fungal flora from coffee*

Samples of fungal DNA extracted from coffee beans produced DNA bands on DGGE gels that had sufficient intensities to be analyzed by sequencing (Fig. 3 and 4). Two reference DNA of *A. carbonarius* and *A. niger* were used as markers and loaded on DGGE gels (bands 1 and 2) to facilitate identification. In Fig. 3 and 4, each vertical line represents a coffee beans sample and each band represents a mould species. The PCR-DGGE patterns of duplicate coffee for each stage of processing were similar and revealed the presence of three to twelve bands for each coffee sample. In addition, the comparative study of strains obtained from both methods suggests a higher diversity in the fungal population obtained by DGGE method than with traditional microbial techniques for the same samples. However, some species that appear in the profiles of direct methods were absent in the DGGE profile (Table 1).

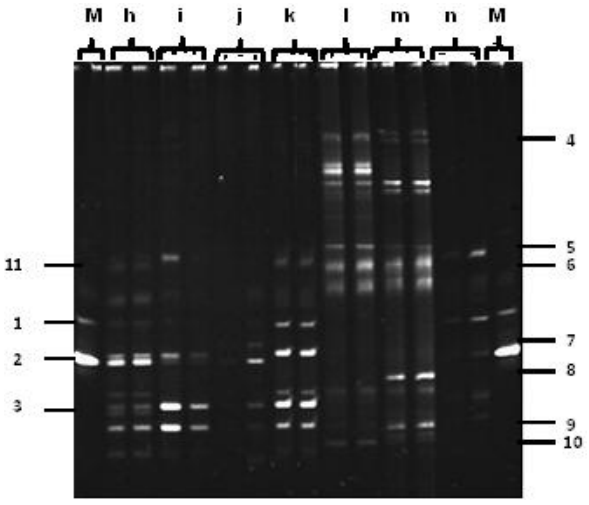


**Fig. 4**. DGGE profiles of PCR amplicons of the domain D1 of 28S rDNA that represent the fungi biodiversity in samples taken during treatment of coffee (M: marker; h-n: samples of coffee (3410, 0510, 1210, 1110, 4210, 4010, and 0410). The position of bands is indicated by numbers that correspond to species of fungi: 1*: A.*

*niger;* 2: *A. carbonarius;* 3: *P. arenicola;* 4: *Mucor sp.;* 5: *Wallemia sebi*; 6: *Acremonium murorum;* 7: *A. ochraceus;* 8: *A. nomius;* 9: *P. citrinum,* 10*: P. roqueforti,* 11: *Wallemia muriae.*

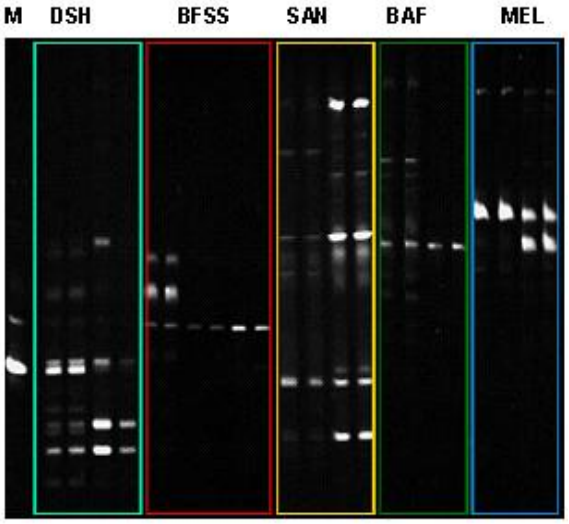


**Fig. 5.** PCR-DGGE 28S rDNA banding profiles of coffee moulds from Dschang (DSH), Bafoussam (BFSS), Santchou (SAN), Bafang (BAF) and Melong (MEL), taken during two treatment (wet and dry process). (M: marker).

*DGGE pattern of moulds DNA from coffee among different locations*

On DGGE gel, the observed bands had sufficient intensities to analyze samples of fungal DNA extracted from coffee, from various geographical locations in Cameroon (Fig. 5), so the total quantity of DNA deposited in the wells of DGGE gel was sufficient to consider that moulds DNA could be used as potential markers to ensure the determination of coffee origin. The reference DNA of *A. cabonarius* and *A. niger* indicates that DGGE was working successfully. Each vertical line represents a coffee and each spot represents a moulds. The PCR–DGGE patterns of duplicate sample of coffee for each location were similar (Fig. 5).

In addition, the dendogram obtained (Fig. 6) shows that at 35.4% of similarity, two main clusters were obtained: the firs cluster included the samples of Bafang, Santchou and Melong while the second cluster comprised the samples of Bafoussam and Dschang. At 48.3% similarity two secondary clusters were formed between the samples of Santchou and those of Melong. High similarity exists thus between the moulds profiles of Santchou and Melong, and high similarity exists thus between the moulds profiles of Dschang and Bafoussam, although distance of aproximatively 48km.

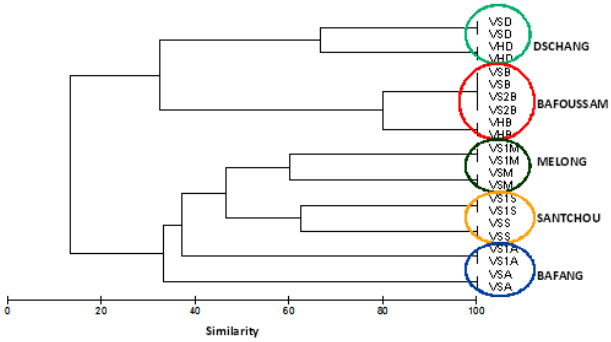


**Fig. 6.** Cluster analysis of 28S rDNA banding profile for coffee fungal communities from five localities (Dschang, Bafoussam, Melong, Santchou and Bafang).

**Discussion**

Confirmation of species identification by DNA sequencing was not carried out in most of the previous studies of fungi on coffee (De Moraes and Luchese, 2003; Martins *et al.*, 2003; Taniwaki *et al.*, 2003) resulting in the possible misidentification of species that were difficult to distinguish by morphology alone. In this study, we analyzed the mycobiota using a molecular approach for global analysis of the DNA of fungi isolated or present in coffee beans.

All fungal genera that we identified had already been recorded in coffee (Mislivec *et al.*, 1986; Abdel-Hafez and EL-Maghraby 1992; Roussos *et al.* 1995). According to Pitt and Hocking (1997) *Aspergillus* competes for substrate with *Fusarium* and *Penicillium* and only increases in incidence in environments with high temperature and low water activity, ideal conditions found in the final stage of processing and drying during storage. Members of the genera

*Penicillium, Fusarium* and *Aspergillus* have been reported to produce all types of pectinase. Occurrence of these filamentous fungi in coffee beans was associated with reduction in the quality of the beverage (Krug, 1940; Bitancourt, 1957 b; Alves and de Castro, 1998). Presence of some filamentous fungi could be relevant for product safety to the extent that, when they are present in foods, they are able to synthesize and excrete mycotoxins that are known cause intoxications. As for coffee, OTA is currently the most watched mycotoxin. Identification of molds based on the sequencing of genes encoding the 28S ribosomal RNA has become a powerful tool to study the communities of molds in environmental samples (Cocolin *et al*., 2002a; Möhlenhoff *et al*., 2001; Li *et al*., 2008). The D1 region of the fungal 28S rDNA gene has also been used in the characterization of fungal population profiles by Cocolin *et al*. (2002a) and El Sheikha *et al*. (2010). The DGGE profiles of most samples were a mixture of prominent bands and others of lower intensity. The intensity an individual band is thought to be a semi-quantitative measure of the abundance of this sequence in the population (Muyzer *et al*., 1993), which suggests that increases and reductions in intensity over the type of process are a reflection of changes in the populations.

PCR-DGGE has proven to be a rapid and effective method to describe the fungal communities of coffee (Masoud *et al*., 2004). In our case, we have shown that for the same sample analyzed with traditional techniques and with PCR-DGGE, those obtained by DGGE showed, in general, a greater microbial diversity. This confirms the idea put forward by Laforgue *et al*. (2009) who showed that PCR-DGGE was an effective and quick method to follow fungal communities of food products. In fact, it is likely that the detection limit of a species in a mixture resulted from the combination of the level of the total population, the number of species and their specific concentration within the mixture. But in any case, it is important to note that the lack of band detection on a DGGE gel species after direct analysis does not necessarily mean their absence in the sample.

PCR-DGGE method was often used but there are still some disadvantages, such as detecting residual DNA that belongs to a species that could be present at some stage on the beans but could disappeared or cannot be cultivated in standard laboratory conditions. That may reduce its effectiveness to analyze fungal communities. Despite these limitations, DGGE is strongly preferred and is considered as one of the best techniques for monitoring the fungal community of a food in a comprehensive, rapid and reproducible way (El Sheikha *et al*., 2010).

The determination of geographical origin is one demand of the traceability during commercial transaction of foodstuffs. One hypothesis of tracing the source of a product is by analyzing in a global way the fungal communities on the food samples. This is based on the hypothesis that the environment has an effect on the fungal ecology of food. The differences in the band profiles can be attributed to the differences in environment between locations. The types of processing system applied could also affect the microbial communities of coffee.

In fact, when comparing the different locations of coffee sampling with the statistical analysis of DGGE pattern throughout the study, we could note that we obtained a complete statistical correspondence between the geographical areas and the fungal communities. We could conclude that there were enough environmental differences between the districts where the coffee were harvested to obtain a major effect on the moulds ecology, whereupon we could create a statistical link between the yeast populations and the geographical area.

**Conclusion**

At the end of this study, the results obtained by the molecular method reflected those obtained by the

classical methods, but the molecular approach has the advantage of rapidity (less than 24h) and specificity. The presence of several fungal DGGE bands showed around 97% homology that culture-independent methods area useful complement to cultured methods to fully characterize the coffee process. In addition, the analysis of coffee fungal communities by PCR–DGGE could be applied to differentiate geographical locations. We showed that the biological markers for the specific locations were sufficient statistically to discriminate regions. This global technique is quicker (<24 h) than all of the classical microbial techniques and avoids the precise analysis of moulds by biochemistry or molecular biology (sequencing). This method can thus be proposed as a rapid analytical traceability tool for coffee and could be considered as a provider of a unique biological bar code.

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