# NOVEL MOLECULAR FINGERPRINTING FOR GEOGRAPHICAL TRACEABILITY OF TIMBER

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**EL SHEIKHA AF, CHALIER C, ZAREMSKI A & MONTET D. 2013. Novel molecular fingerprinting for geographical traceability of timber.** Traceability is defined according to ISO 9000 as the ability to retrieve the origin and use of an article or an activity through a registered method. Its implementation in the timber industry is delayed because of limits of classical identification systems with regard to the nature of timber and features of the manufacturing process. One hypothesis of tracing the source of timber and its products is by analysing in a global way the microbial communities of timber and linking this analysis statistically to its geographical origin. We proposed a very innovative tool of fungi ecology, the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), that was used to characterise the fungi flora of two tropical timbers species, teak and limbali from four countries: Côte d'Ivoire, Cameroon, Central African Republic and French Polynesia. The aim was to show if there was statistical relation between the fungal communities of the timbers and their geographical origins. PCR-DGGE method is a new, simple and cheap traceability tool that can trace the original locations of timbers.

Keywords: Tropical timber, PCR-DGGE, fungi communities, geographical origin

**EL SHEIKHA AF, CHALIER C, ZAREMSKI A & MONTET D. 2013. Pemprofilan molekul yang baharu bagi pengesanan geografi kayu.** Mengikut piawai ISO 9000, pengesanan ditakrifkan sebagai kebolehan mencari asal usul dan kegunaan sesuatu barangan atau aktiviti melalui kaedah yang dikenal pasti. Implementasi pengesanan dalam industri kayu lambat disebabkan kekangan sistem pengecaman klasik tentang ciri-ciri kayu dan proses pengeluaran. Satu hipotesis bagi mengesan sumber kayu dan produknya adalah dengan menganalisis komuniti mikrob kayu secara global dan menghubungkaitkan analisis ini secara statistik kepada geografi asalnya. Kami mencadangkan satu alat inovatif melibatkan ekologi kulat iaitu tindak balas rantai polimerase-elektroforesis gel cerunan nyah asli (PCR-DGGE) yang digunakan untuk mencirikan flora kulat pada dua jenis kayu tropika iaitu kayu jati dan limbali dari empat negara iaitu Côte d'Ivoire, Cameroon, Republik Afrika Tengah dan French Polynesia. Tujuan kajian adalah untuk menyelidik sama ada terdapat hubungan antara komuniti kulat kayu dengan geografi asalnya. Kaedah PCR-DGGE merupakan alat yang baharu, mudah digunakan dan murah bagi mengesan lokasi asal kayu.

## INTRODUCTION

Traceability of products to their origin has been developed recently, especially in food and other economical industries. The capacity to perform a complete follow-up of products in industries has been possible with the implementation of automatic identification systems, which are able to establish a link between the products, origin of the products and the process. The follow-up process is named traceability and many technical solutions have been developed to carry it out, e.g. sticky labels and bar codes. However, these methods are only administrative and in case of disputes over geographical origin, they cannot solve the problem. To date, there is no analytical tool that permits precise identification of geographical origin.

Timber is an important source of income for many tropical countries. Local and indigenous people depend on tropical forests for their livelihood. Most tropical timbers have good

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natural durability, rendering them suited for the outdoor environment without impregnation with preservatives. In addition, many tropical timbers have aesthetic qualities and great strength (Montagnini & Jordan 2005).

Currently, traceability systems such as bar code and radio frequency label use markings (pencil, paint, ultraviolet or mechanical) as digital fingerprints (Chiorescu & Berg 2003, Fuentealba et al. 2006) or internal signatures (Choffel & Charpentier 1999). The complete techniques suitable for timber follow-up are listed by Fuentealba et al. (2006). However, these methods are limited to certain parts of the life cycle of the timber and, consequently, cannot certify the geographical origin of timber. Strontium isotopes are used to determine the geographical origin of tropical timber but this method is expensive and the data bank needs to be often updated (Havard et al. 2007). The possibility of using DNA of timber as markers to verify the timber source poses many problems (Lowe 2007, Nielsen & Kjær 2008, Tnah et al. 2009, Finkeldey et al. 2010).

The microbial flora have been found to be specific for each place of production in the case of fruits based on yeast and fungal DNA (El Sheikha 2010, 2011, El Sheikha et al. 2009, 2011). The same was observed in aquaculture fish using bacterial DNA (Le Nguyen et al. 2008). Microbial flora can be a biological marker, allowing for identification of geographical origin and quality control of various products using the diversity of microbial communities present in the products.

Dry wood is generally composed of cellulose, lignin, hemicellulose and minor amounts (5 to 10%) of extraneous materials. Cellulose, the major component, constitutes approximately 50% and hemicellulose constitutes 25% of the wood substance (w/w). Lignin constitutes 23 to 33% of the wood substance in softwood and 16 to 25% in hardwood. Although lignin occurs in wood throughout the cell wall, it is concentrated towards the outside of the cells and between cells. Lignin is often called the cementing agent that binds individual cells together (Miller 1999). Timber contains hydrocarbon substances necessary for the growth of fungi especially lignivorous fungi and can be altered by them in the presence of moisture above 20% and temperature above 24 °C. These conditions are common in tropical regions (Rossmoore 1995). Wood decay is also related to fungal ecology. This raises an interesting question about the fungal species causing wood decay (Parfitt et al. 2010). The hypothesis of this work was to link the ecology of fungi in general and lignivorous fungi in particular to geographical origin. It was thus necessary to identify and establish a specific genetic fingerprint of the different species of fungi present on timber.

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 timber. This method is based on the assumption that the microbial communities of products are specific for a geographical area (Le Nguyen et al. 2008, Montet et al. 2008, El Sheikha et al. 2009).

The main objective of this study was to apply polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) method to analyse all fungi present on or in the timber to create an analytical technique that would permit linkage of fungi communities to geographical origin. The acquired band patterns for fungi communities of different species of timbers and locations were compared and analysed statistically to determine the geographical origin of the timber. To the best of our knowledge, this is the first research applied to timber describing PCR-DGGE, a molecular method of origin analysis. It is a simple, rapid and cheap technique compared with isotopic techniques. PCR-DGGE permits the certification of timber origin in general and tropical timber in particular using 28S rDNA fingerprinting of fungi.

## MATERIALS AND METHODS

### Biological material

Timber samples were collected and identified using anatomical, physiological and biochemical characteristics. Four tropical timber samples were collected from different districts in four different countries: teak 16476 (*Tectona grandis)* from Bouaké in Côte d'Ivoire, teak 34090 (*T. grandis*) from Bambuko in Cameroon, limbali 18865 (*Gilbertiodendron dewevrei* and *G. preussii*) from Berbérati in Central African Republic and limbali 5473 (*G. dewevrei* and *G. preussii*) from Nuku Hiva in French Polynesia. The main characteristics of teak and limbali are presented in Table 1.

### Site

Bouaké of Côte d'Ivoire has humid tropical climate. The northern climate is more varied

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than the south and the temperature range is higher, 22 to 35 °C. Sunlight is constant and the humidity is lower than in the south. Bambuko has wet evergreen forest (rainfall 2500 mm annually). The soil of recent volcanic origin is more or less deep and relatively fertile (superficial soil covered with savannah). The soil experiences poor water penetration. Berbérati in Central African Republic has temperate climate (24 to 36 °C). The average annual precipitation is 1157 mm. The average relative humidity is around 78%. In French Polynesia, Nuku Hiva has subtropical climate with large local variations in rainfall (1000 to 3000 mm annually). The wettest season is the middle of the year. Average temperature is 26 °C.

### Molecular technique

The method was based on DNA extraction of all fungi present on or in the timber using the method of El Sheikha (2010). A single PCR was then performed using a universal primer for all fungi DNA. A fragment of the region of the 28S rDNA gene was amplified. The amplified fragment was 260 base pairs (bp) (Wu et al. 2002, Li et al. 2008). A 30-bp GC-clamp was added to the forward primer in order to ensure that the fragment of DNA remained partially double-stranded and that the region screened was in the lowest melting domain (Sheffield et al. 1989).

The PCR products were analysed by DGGE using a universal mutation detection system as first described by El Sheikha (2010). The gels were stained and photographed. Images were statistically analysed after spot alignment using a software image analysis. The DGGE profile thus created is considered as an image of all the main fungi present in the samples: a band corresponding to a single type of sequence or phylotype or fungus. The photo represents a bar code. Significant difference in fungi communities of timber samples from different origins was determined using factorial correspondence analysis based on two variances that described most of the variation in the data set.

## RESULTS

### Efficiency of the new protocol for extraction of fungal DNA from tropical timber

DNA extraction of the fungi community was conducted on tropical timber samples using two protocols, namely, the first one developed by Le Quéré et al. (2002) and the second improved by us. By comparing the results of DNA extracts by BioSpec-nano UV-VIS spectrophotometer for the two protocols (Table 2), we found that the quality of DNA from our protocol (new one) was effectively better than the other protocol but the quantity of DNA was smaller.

**Table 1** List of studied timber species

| Reference no. | Commercial name | Scientific name | District | Country |
|---|---|---|---|---|
| 16467 | Teak | *Tectona grandis* | Bouaké | Côte d'Ivoire |
| 18865 | Limbali, Vaa | *Gilbertiodendron dewevrei, G. preussii* | Berbérati | Central African Republic |
| 5473 | Limbali, Vaa | *Gilbertiodendron dewevrei, G. preussii* | Nuku Hiva | French Polynesia |
| 34090 | Teak | *Tectona grandis* | Bambuko | Cameroon |

**Table 2** Quantity and purity of the extracted DNA by testing two protocols for fungal DNA extraction

| Sample | Le Quéré et al. (2002) | | This study | |
|---|---|---|---|---|
| | Nucleic acid (ng $\mu L^{-1}$) | OD 260/280 nm | Nucleic acid (ng $\mu L^{-1}$) | OD 260/280 nm |
| Teak 16467 | 34.72 | 1.32 | 10.31 | 2.03 |
| Limbali 18865 | 81.28 | 1.37 | 20.14 | 1.95 |
| Limbali 5473 | 47.04 | 1.27 | 20.02 | 1.83 |
| Teak 34090 | 72.7 | 1.25 | 14.99 | 2.11 |

OD = optical density

## Verification of the PCR amplification of the extracted DNA

The fungal DNA obtained after extraction was amplified by classic PCR with a protocol improved by us. In order to verify the efficiency of this fraction, the PCR amplicon was electrophoresed. All bands were clearly observed and had a molecular weight of 260 bp, the expected size of the amplicon. The intensity of bands representing the PCR amplicons was important. Fungal DNA was amplified very well and thus it was possible to continue the analysis of these amplicons by DGGE method.

## DGGE pattern of fungal DNA from tropical timbers from different countries

On DGGE gel, the observed bands had sufficient intensities to analyse samples of fungal DNA extracted from tropical timbers from various geographical areas (Figure 1). Therefore, the total quantity of DNA deposited in the wells of DGGE gel was sufficient to consider that fungal DNA could be used as potential markers to determine timber origin. The reference DNA of *Mucor racemosus* and *Trichoderma harzianum* indicated that DGGE was successful. Each vertical line represented a timber sample and each spot,

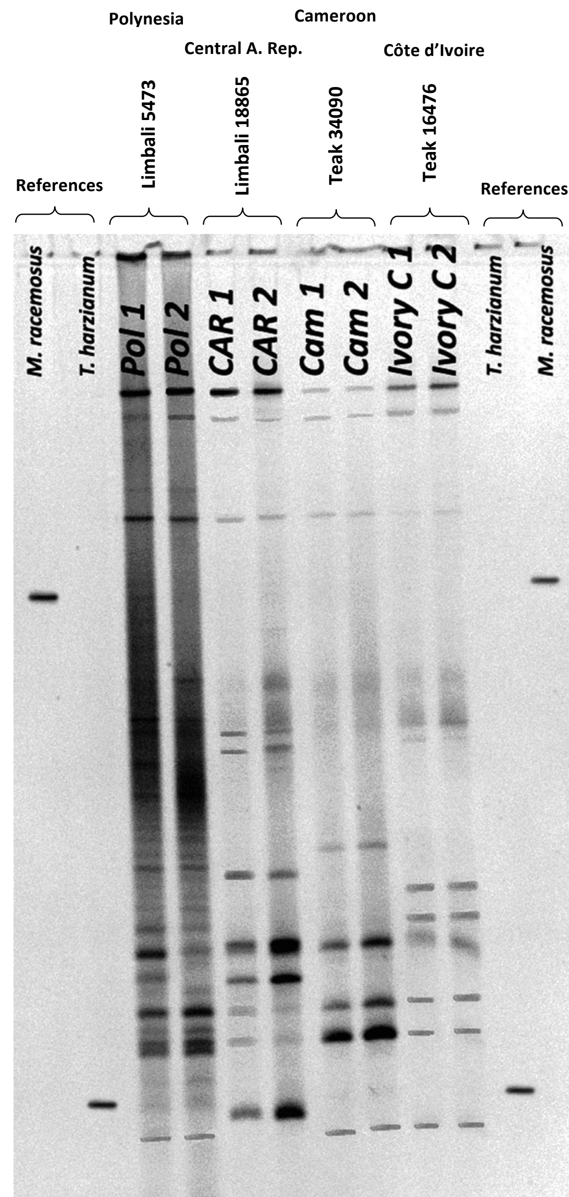


**Figure 1** PCR-DGGE of 28S rDNA band profiles of different varieties of tropical timbers from four countries: Côte d'Ivoire (Ivory C), Cameroon (Cam), Central African Republic (CAR) and French Polynesia (Pol); 1, 2 = duplicate timber samples from the same country

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a fungus. The PCR-DGGE patterns of duplicate tropical timber for each location were similar for each country and revealed the presence of 8 to 25 bands for each tropical timber sample (Figure 1).

Factorial correspondence analysis proved to be a useful statistical tool to compare the similarity of the fungi communities of tropical timber samples from four different countries. For the timber samples, the two variances were 89% between the fungal communities (Figure 2). There were clearly four different groups for four different countries.

## DISCUSSION

We proposed a new protocol that optimised fungal DNA extraction from timber samples. We adapted this protocol to link fungal communities to different geographical origins of timbers by applying PCR-DGGE.

The traceability tool was applied to analyse tropical timbers from different locations. It showed some significant differences in the migration patterns on the DGGE gel. However, duplicates for each sampling location gave statistically similar DGGE patterns throughout the study. The differences in the band profiles could be attributed to differences in the environment between districts. The types of process applied could also affect microbial communities of timbers. In the gel, some common bands appeared in all samples regardless of the location and variety. These bands could be common fungi for all the tropical timber samples.

When we compared the different locations of timber sampling with statistical analysis of DGGE pattern, a complete statistical correspondence between geographical areas and fungal communities was observed. Environmental differences between districts where the timber were collected were enough to cause a major effect on the fungi ecology. Thus, statistical link between fungi populations and geographical areas was evident.

## CONCLUSIONS

The analysis of tropical timber fungi communities by PCR-DGGE could be applied to differentiate geographical locations. Biological markers for

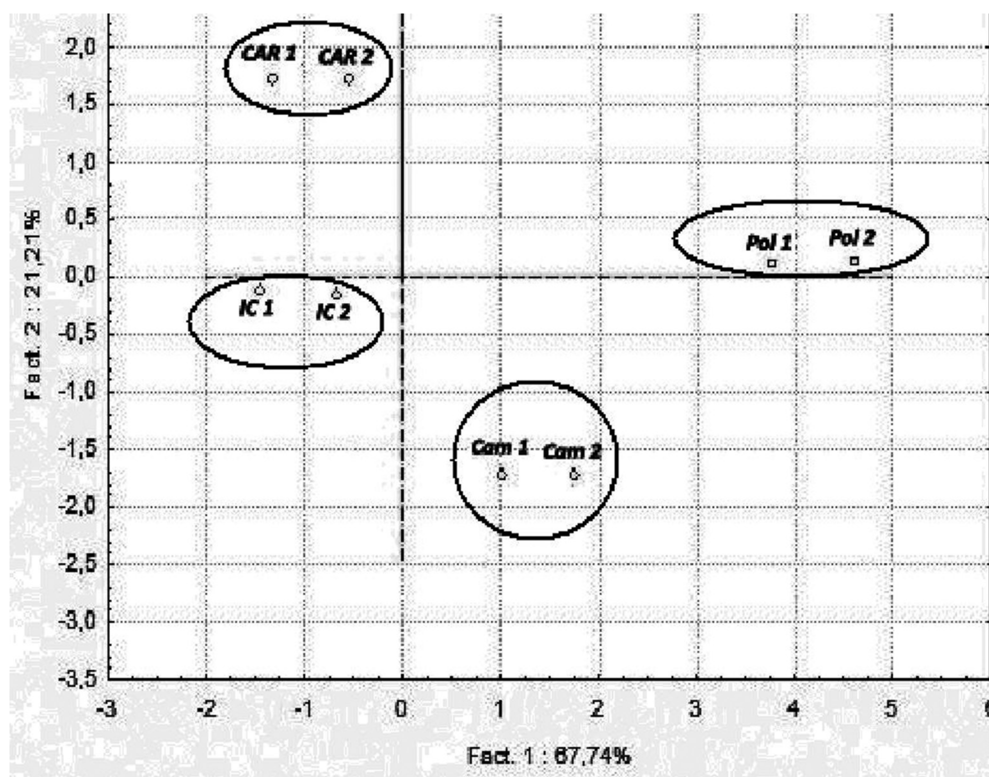


**Figure 2** Factorial variance analysis of 28S rDNA banding profiles of different varieties of tropical timber from four countries Côte d'Ivoire (IC), Cameroon (Cam), Central African Republic (CAR) and French Polynesia (Pol); 1, 2 = duplicate timber samples from the same country

specific locations were sufficient statistically to discriminate regions. This global technique is quick (less than 24 hours) and does not require precise analysis of fungi by biochemical or molecular biology (sequencing) method. This method is a cheaper and more accurate technique compared with isotopic methods. PCR-DGGE method is also a rapid analytical traceability tool for timber and can be considered a provider of unique biological bar code for geographical origin.

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