UMR 95 QUALISUD

Integrated approach for food quality

Dynamics and Biodiversity of microorganisms (fungi, bacteria, yeast) linked to origin and post harvest treatments on coffee beans

DURAND, Noël[*], EL SHEIKHA, Aly[**], SUAREZ-QUIROZ, Mirna-Leonor[***], GONZALEZ-RIOS, Oscar[***], **MEILE, Jean-Christophe[*],** NGANOU DONKENG, Nadège[****], GALINDO-SCHORR, Sabine[*], FONTANA Angélique[*], PAVON, Carmen[***], ESTRADA, Erik[***], MACIA, Isabel[*****], MARTINEZ, Amaury[******], MONTET Didier[*].

*. UMR Qualisud (CIRAD, Université Montpellier II), 34095 Montpellier Cedex 5, France.
**. Department of Food Science and Technology (Minufiya University, Faculty of Agriculture), 32511 Shibin El Kom, Egypt.
***. Unidad de Investigación y Desarrollo en Alimentos, Instituto Tecnológico de Veracruz, 91860 Veracruz, Mexico.
****. Department of Food Science and Nutrition, Food Microbiology laboratory, National School of Agro-Industrial Sciences, University of Ngaoundere, B.P. 455 ENSAI, Cameroon.
*****. Universidad UNELLEZ, Guanare, Venezuela.
******. Universidad UCB, Caracas, Venezuela.

Introduction

- This work aimed at studying the microbial flora associated to different methods of coffee processing

- Our objective is to understand the dynamics of microbial populations linked to post harvest treatments & origins of coffee production

- Large study realized on coffee samples from Mexico, Cameroon and Venezuela

Introduction

The microbial diversity associated with humid process (wash, semi-wash) and dry processing was evaluated on samples of *Coffea arabica L.* which were collected during different post harvest processing stages in Mexico

Dynamic of microbial populations according to three different post-harvest treatments

- 3 farms (fincas) of Mexico
- on Coffea Arabica
- 3 different treatments :

Andrade: mechanical humid process
Jocutla: humid process with fermentation under water
Zongolica: dry fermentation

Postharvest treatment : humid process

Harvest

Cherries

Depulping: Using a pulping machine, which combines the use of friction and a water jet or with blades, separating the grain from the pulp

Depulped coffee

Pulping : After fermentation or mechanical pulping in aqueous medium, the grains are surrounded by a viscous mucilage which is eliminated by fermentation or by chemical action of lime, or water jets

Demucilaginated or fermented coffee

Sun drying or artificial drying. Coffee is in parch

Parchment coffee

Dehulling: Before exportation, coffee is dehulled: the parchment is removed for washed coffee

Green coffee

Postharvest treatment : dry process

Harvest

Coffee Cheries

Drying
Sun or artificial drying

Husk coffee

Dehusking
Before exportation, coffee is dehusked, husk is removed to obtain green coffee

Green coffee

Strategy

- The dynamics and biodiversity of microbial populations (fungi, yeast and bacteria) on coffee beans were monitored using PCR-DGGE (Polymerase Chain Reaction - Denaturing Gradient Gel Electrophoresis)
- Culture-independent method
- Global analysis of the microbial ecology at the molecular level (DNA)
- Provides a snapshot of the microflora composition

➢ **Our objective is to understand the dynamics of microbial populations linked to post harvest treatments, origins of coffee production, by comparative analyses of DGGE fingerprints**

Methods :

PCR-DGGE (Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis)

Coffee sample

1) Extraction of total DNA
microbial DNA and Food DNA
(yeast, fungi, bacteria)

2) PCR Amplification of a ribosomal DNA variable region by specific primers

28S fungi | 16S bacteria | 26S yeasts

3) Separation of different DNA by DGGE

Fungi | Bacteria | Yeasts

DGGE profiles = specific biological barecode

DGGE profiles analysis

DGGE migration

DGGE gel photographed

CCD camera

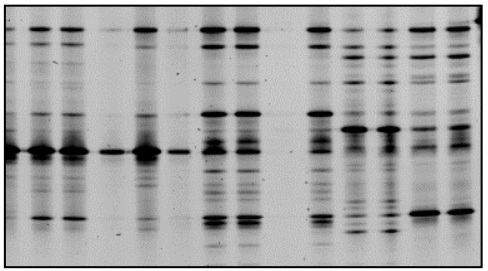
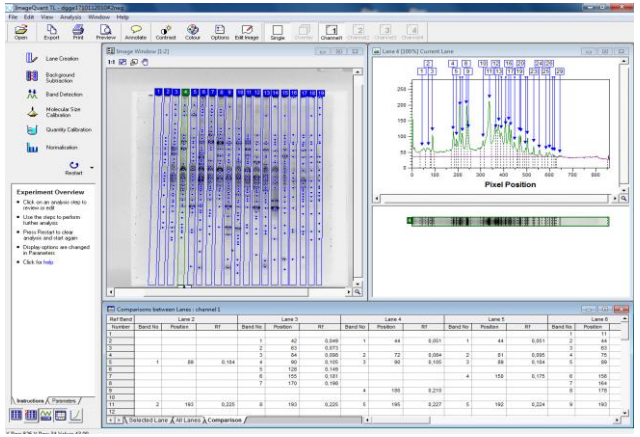
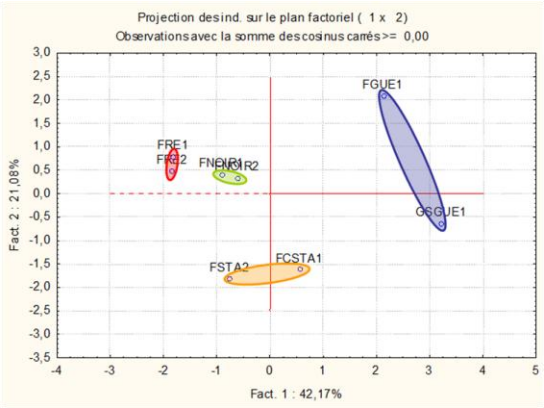


Image Analysis

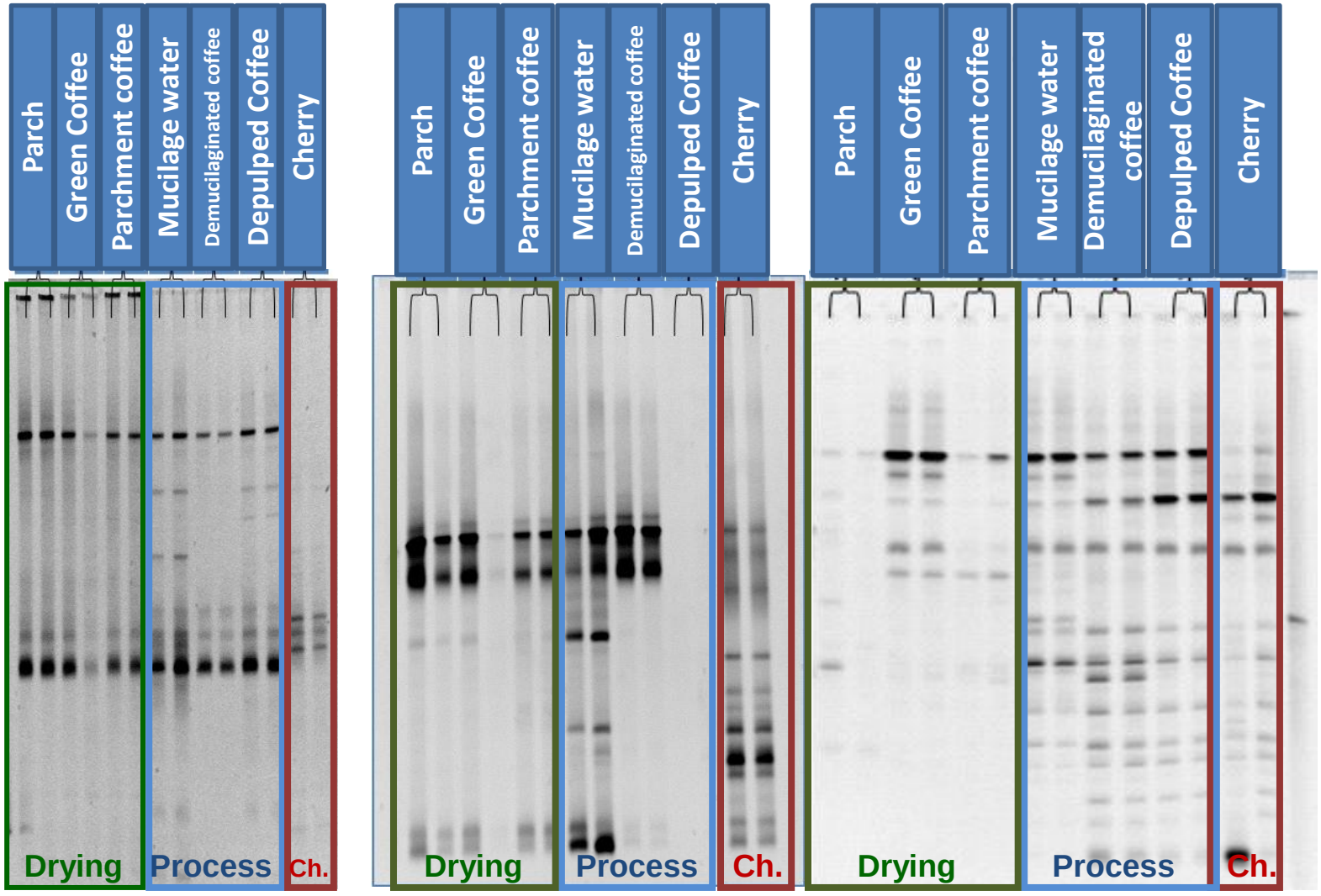


Statistical Analysis
(PCA)

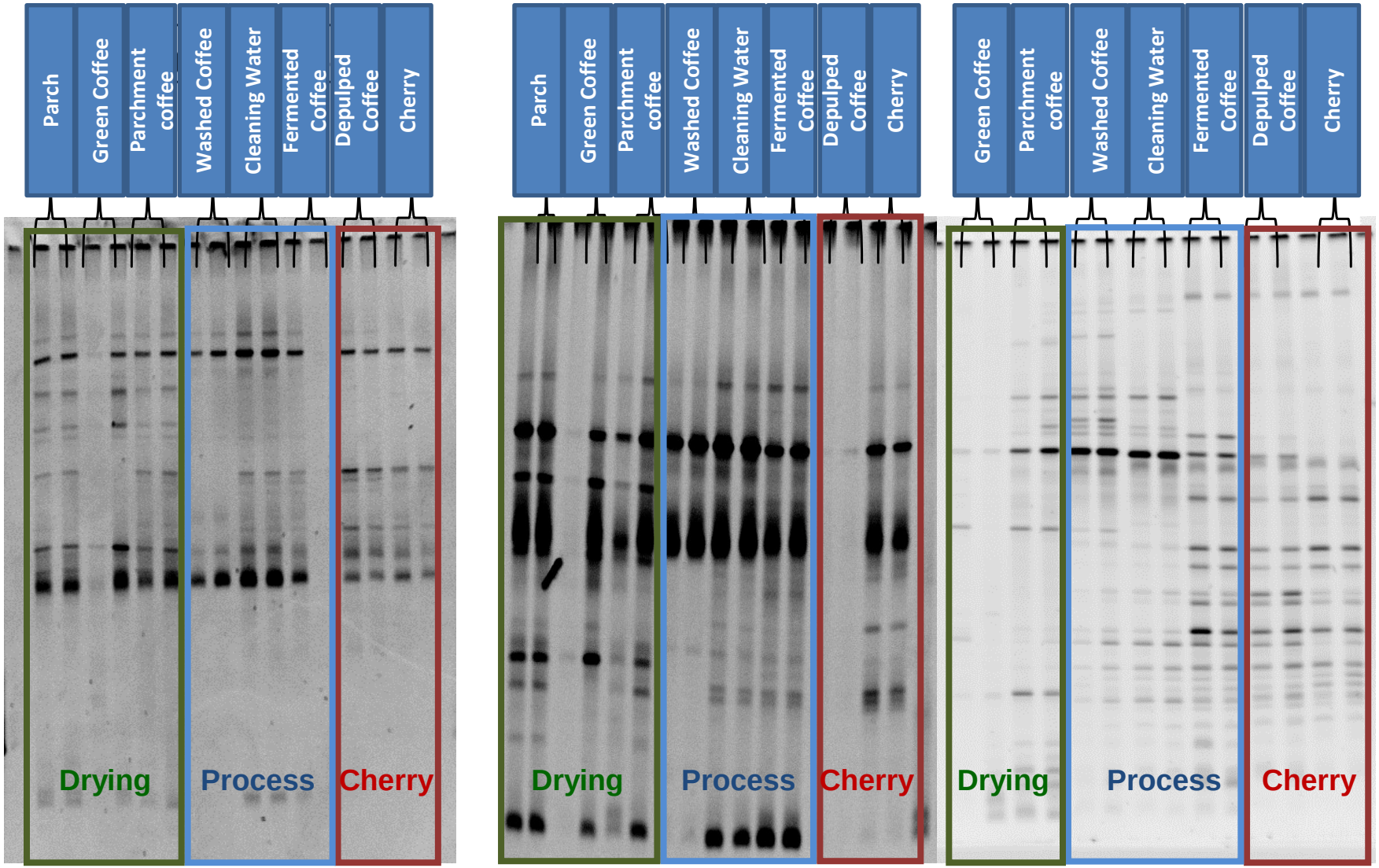


Comparative analysis of profiles linked to specific areas/treatments

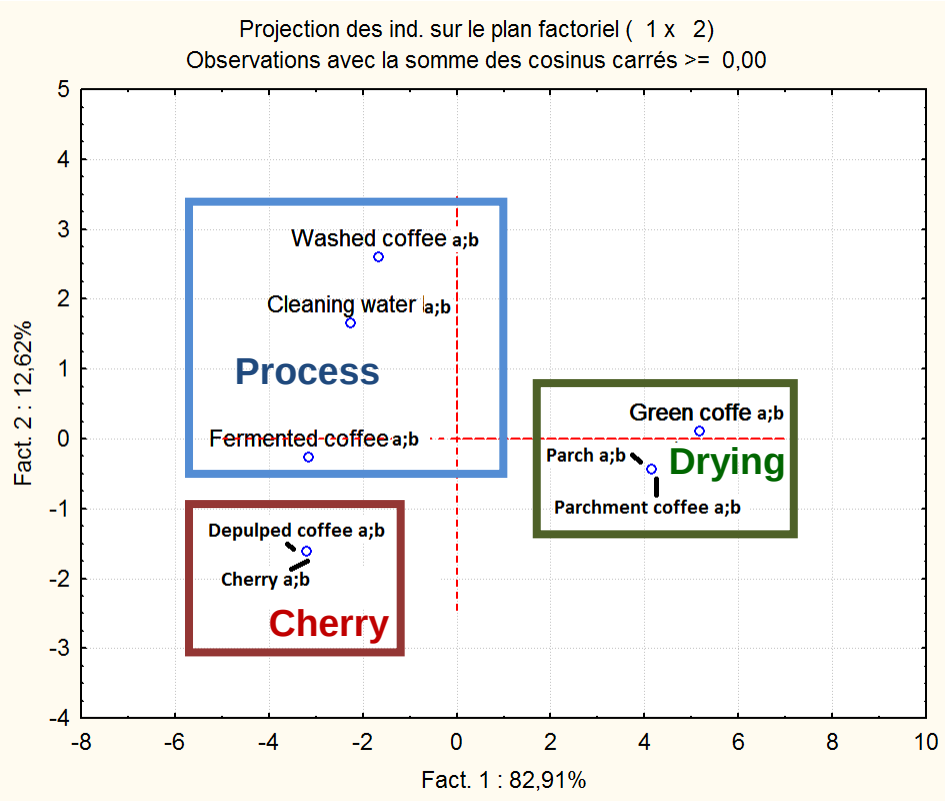
Andrade: Mechanical Humid process



Jocutla : humid process with fermentation under water

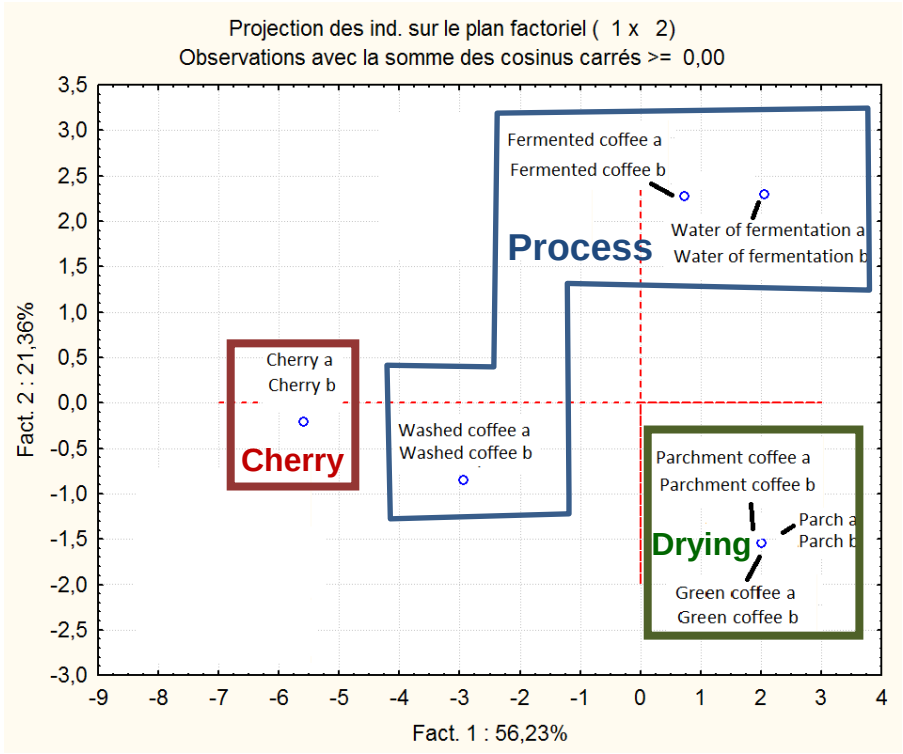


Statistical analysis of DGGE Profiles (Humid Process Finca Jocutla Wash)

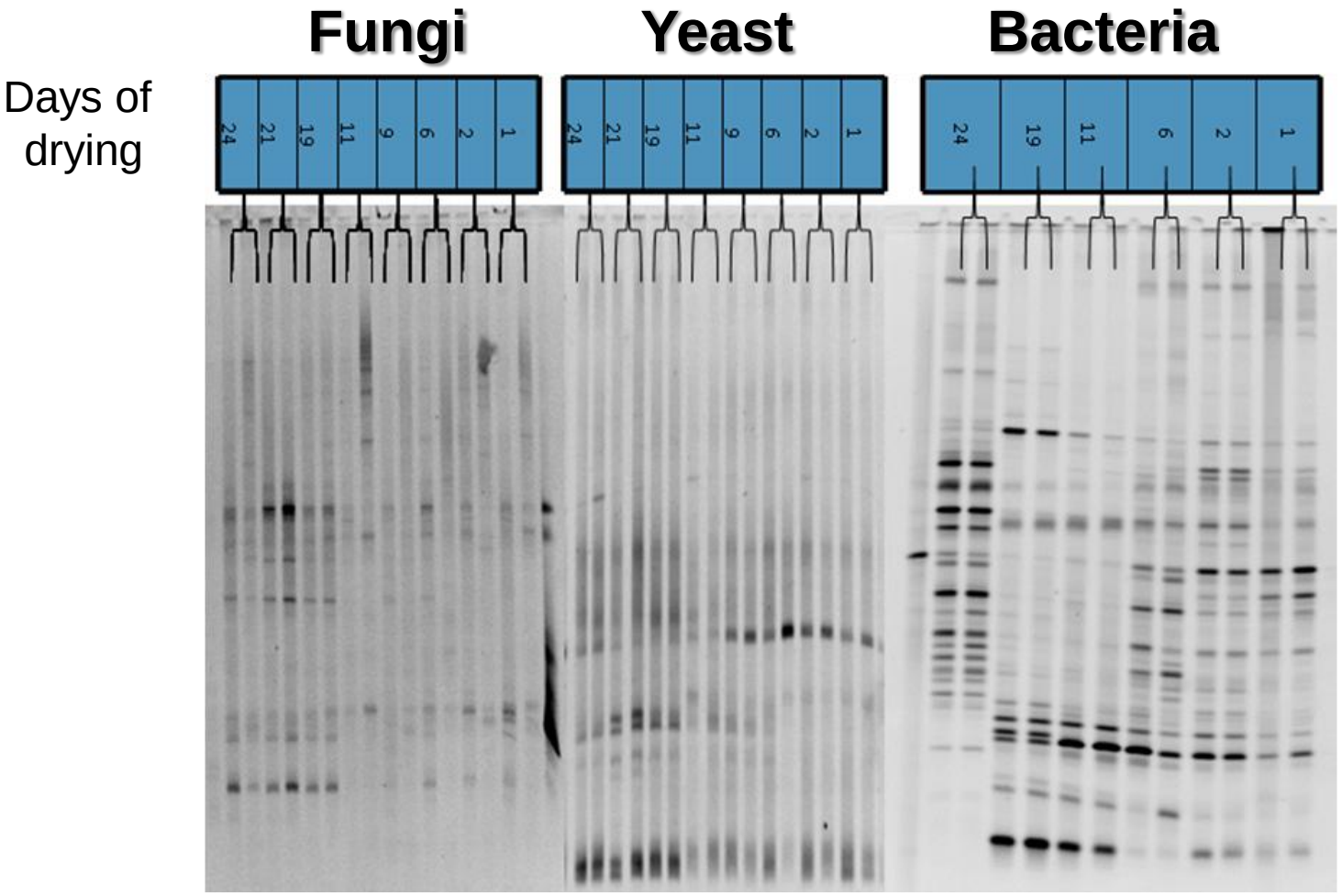


Fungi

Yeast

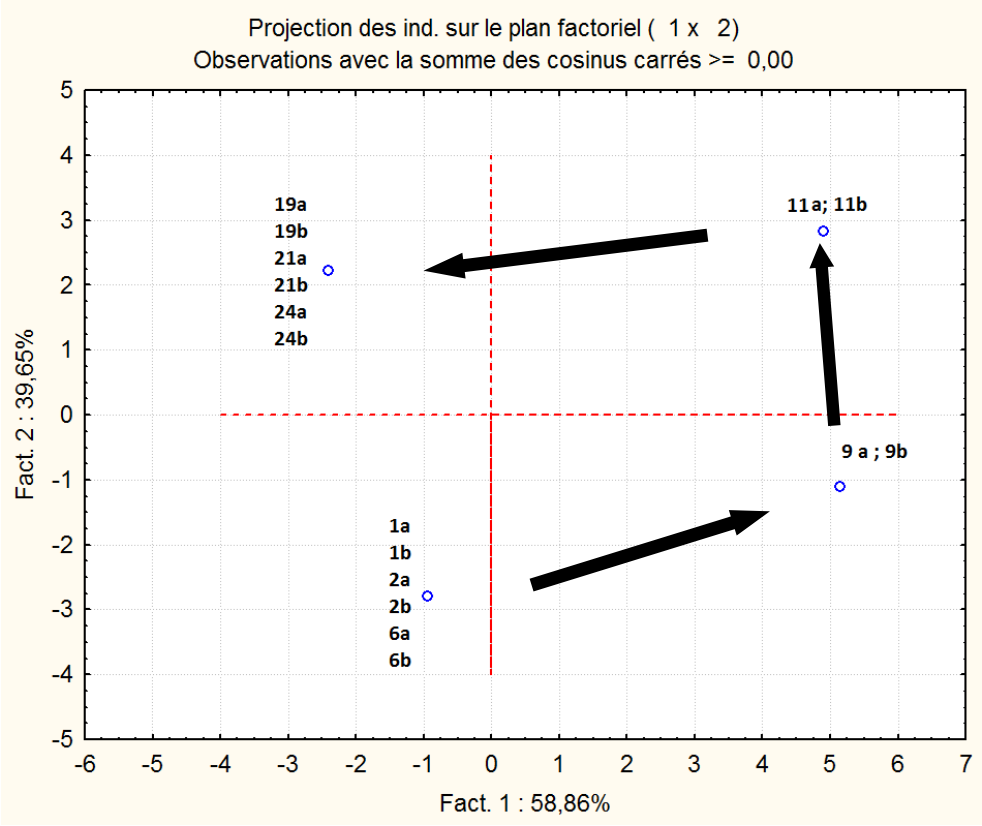


Zongolica : Dry process



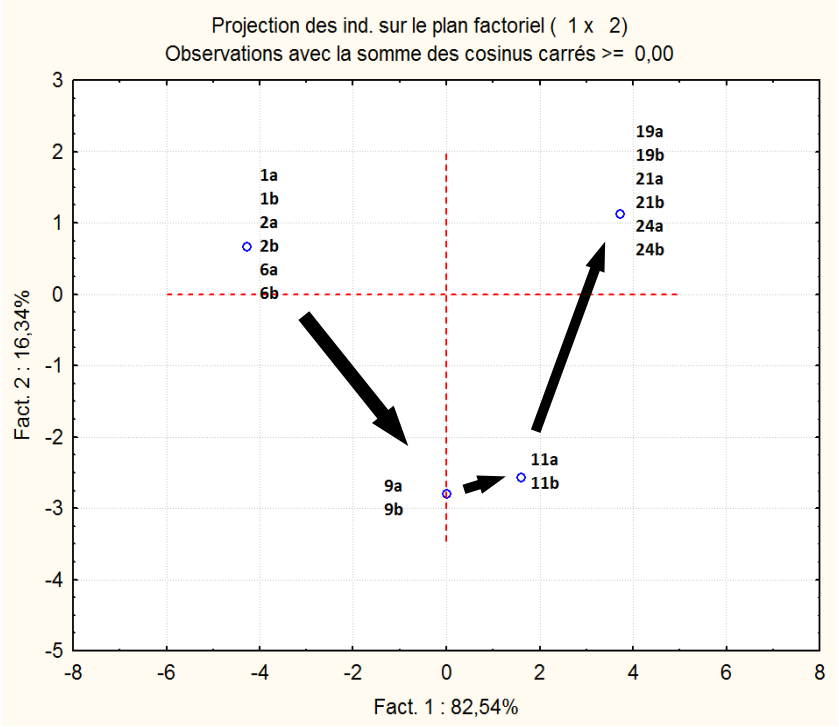
Statisctical analysis of DGGE Profiles

Dry process



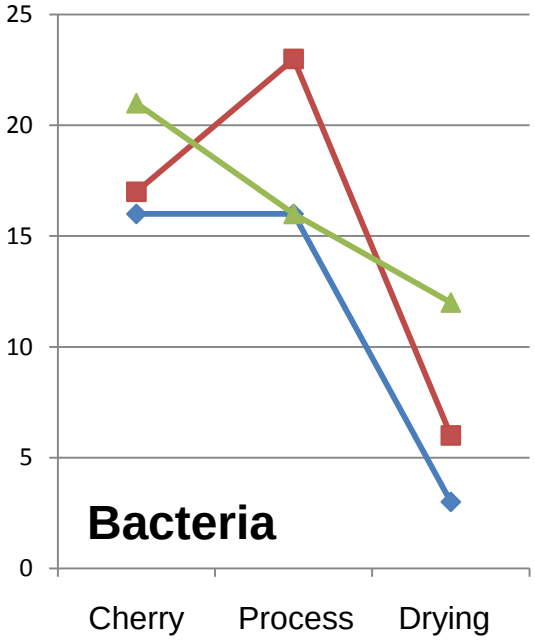
Fungi

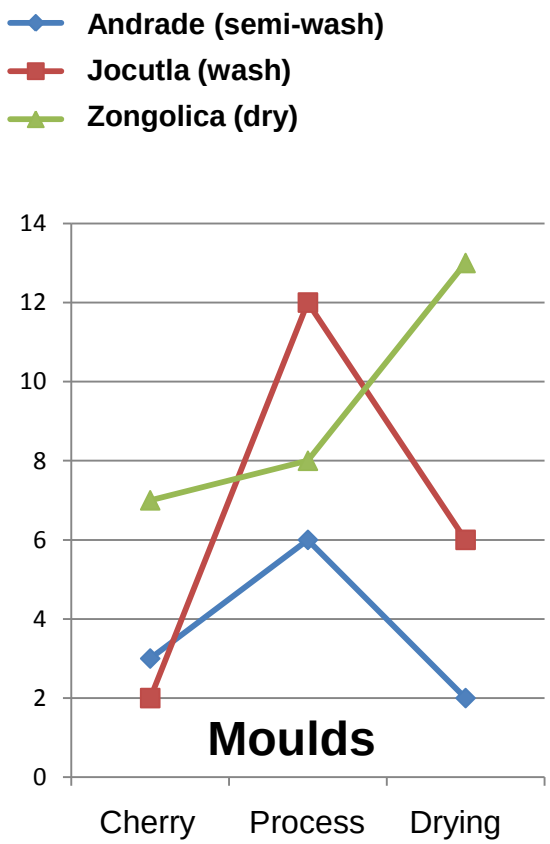
Yeast



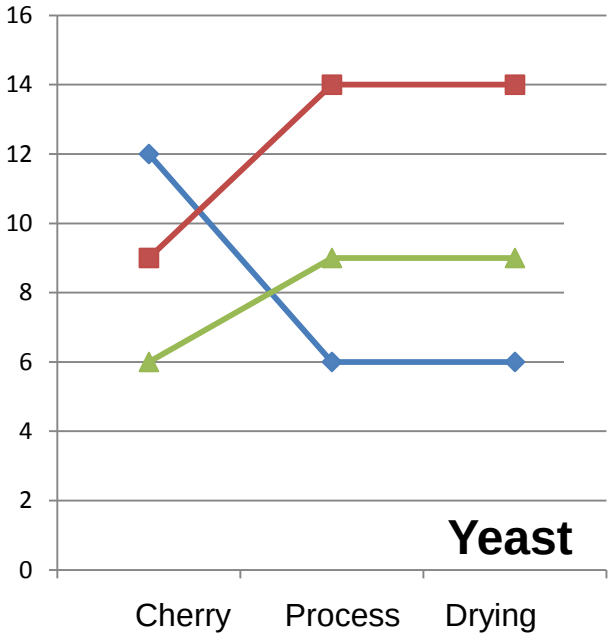
Dynamics of microbial flora during coffee treatments

Number of species

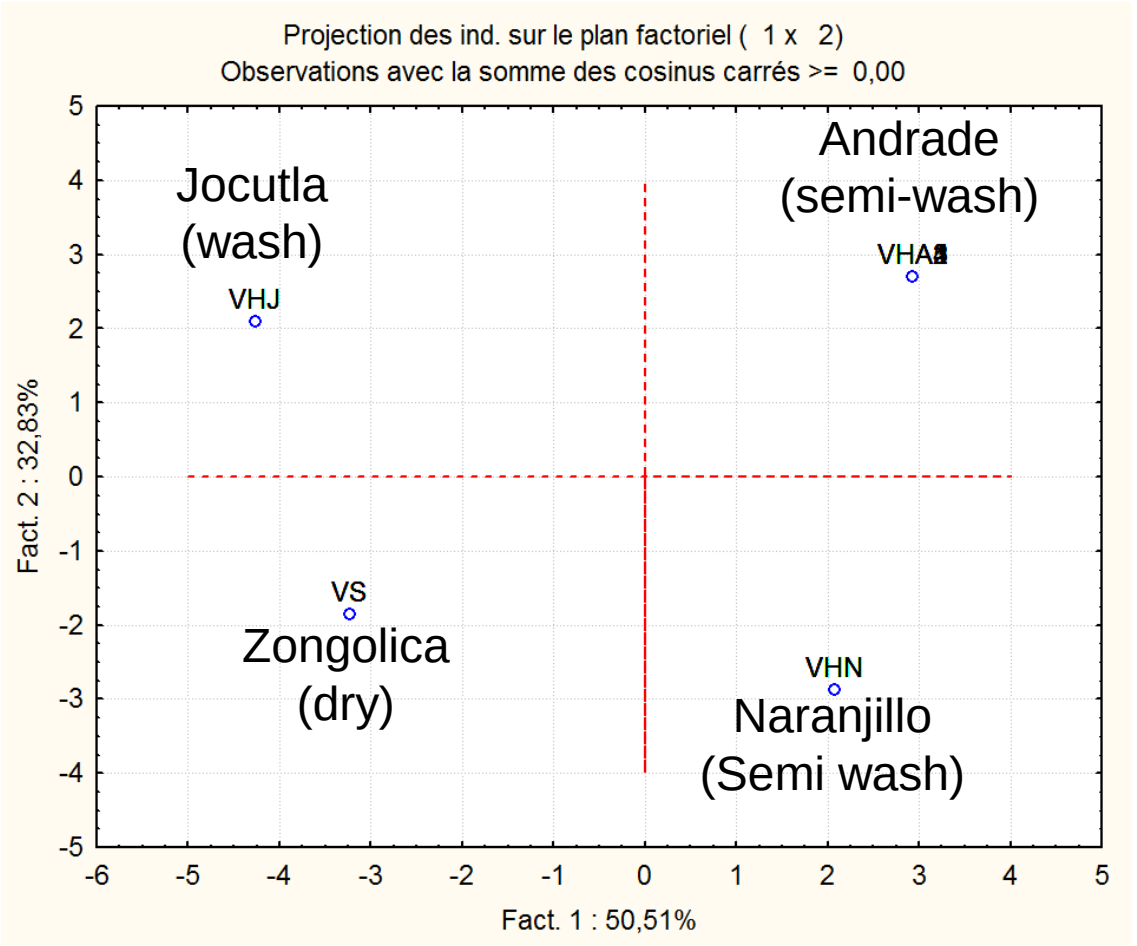




Andrade (semi-wash)
Jocutla (wash)
Zongolica (dry)



Discrimination of Fincas



Mains Conclusions

- The technique used allow a qualitative and semi-quantitative monitoring of the biodiversity and dynamics of microbial populations along the different post-harvest coffee treatments
- We could differenciate the post-harvest steps by using microbial DGGE profiles for three different type of treatments
- The microbial flora structures evolves during treatments and can be subdivided into 3 main classes (field, process, drying)
- We could discriminate bewteen four different mexican fincas using DGGE profiles from green coffee samples
- Differences are probably due to the type of treatment as well as to the geographical origin

Perspectives

- Understanding the interactions between microorganisms associated with
  - Flavour
  - OTA production

- Traceability of coffee (linked post-harvest treatment and geographical origin)
  - Determination of specific signatures
  - Identification of biological markers

Acknowledgements

UMR Qualisud, Montpellier, France

DURAND Noël
EL SHEIKHA Aly
MEILE Jean-Christophe
GALINDO-SCHORR Sabine
FONTANA Angélique
MONTET Didier

National School of Agro-Industrial Sciences, University of Ngaoundere, Cameroon

NGANOU DONKENG Nadège

ITV, VeraCruz, Mexico

SUAREZ-QUIROZ Mirna-Leonor
GONZALEZ-RIOS Oscar
PAVON Carmen
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Acknowledgements

Questions ?

Not good

Good

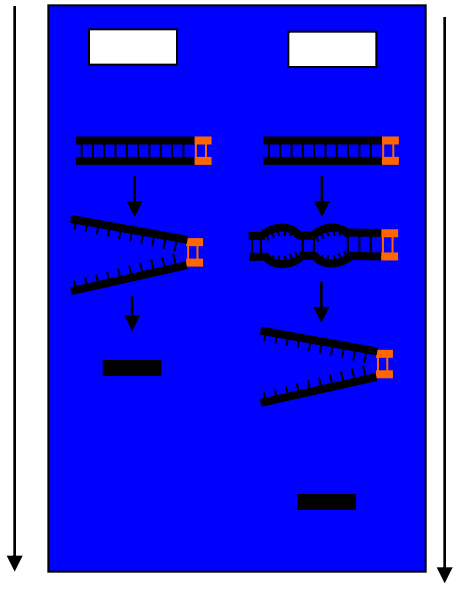
Methods

Double strand DNA of the same size from PCR

DNA lessrich in GC

One band = one strain or one clone

DNA mostrich in GC



Least concentrated in denaturing agents

Linear gradient of denaturings Agents (urea/formamide)

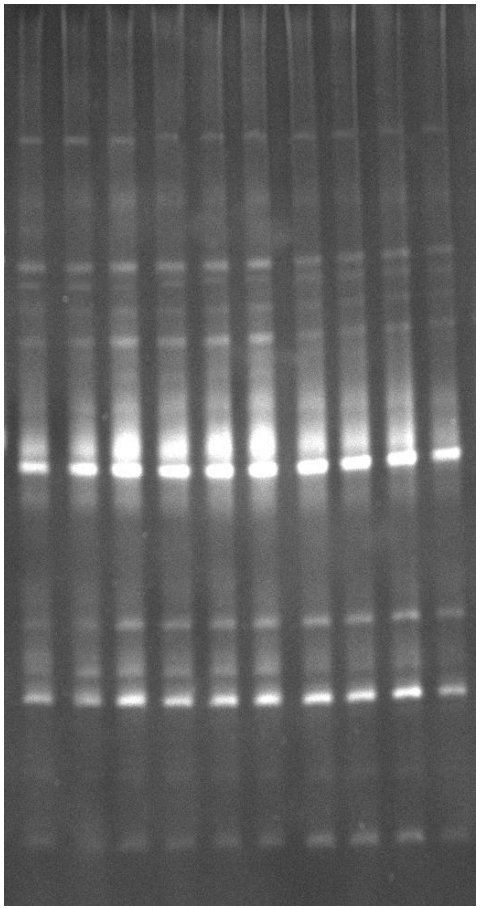
Most concentrated in denaturing agents

Repetability

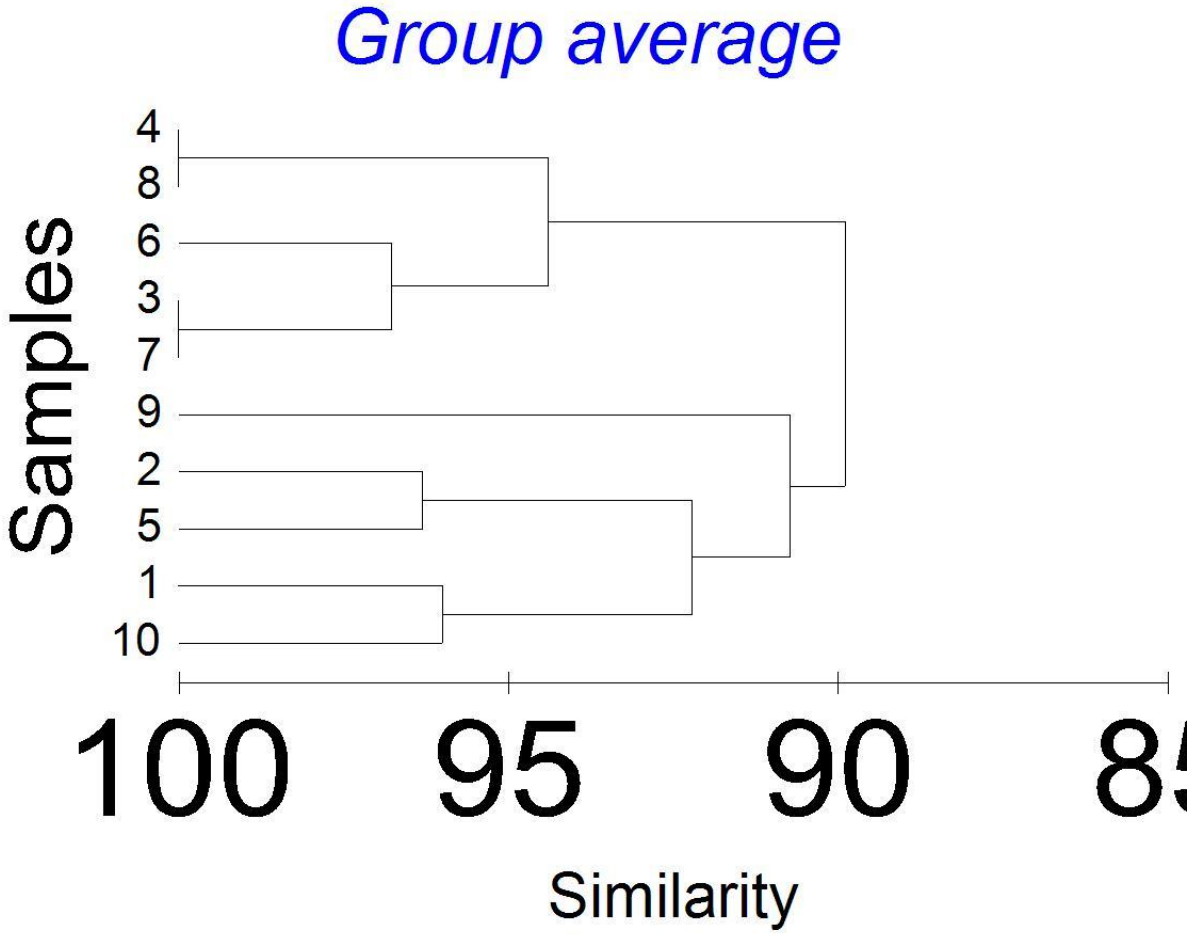
10 samples from the same batch

Coffee Ivory Coast

- Extraction
- Purification
- PCR
- DGGE



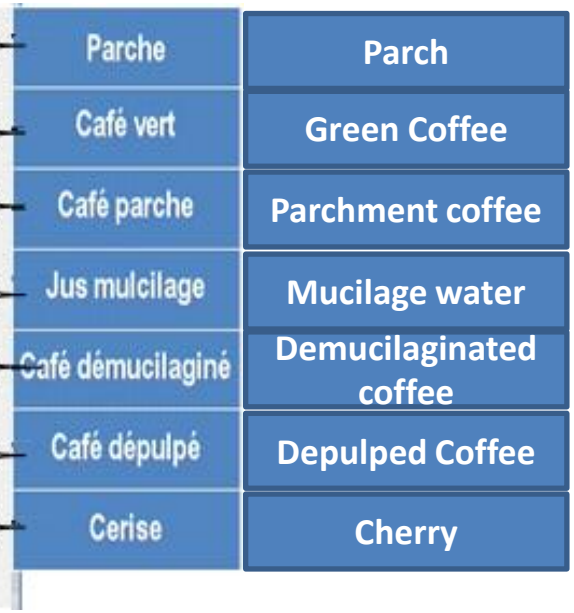
Repetability

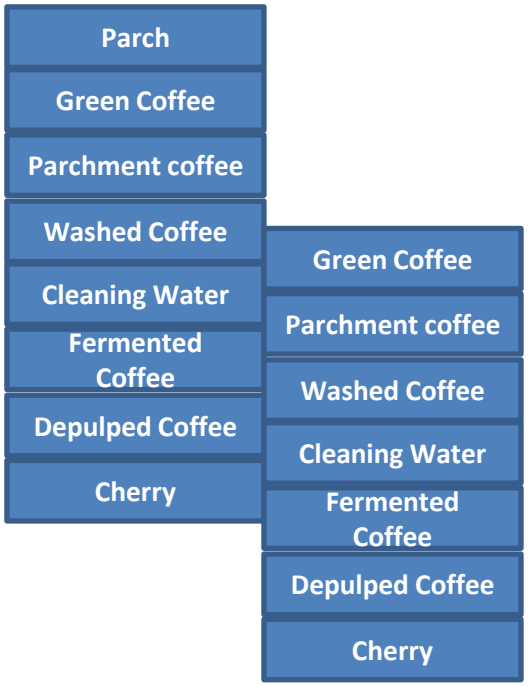


Method sensitivity

- 3 species of fungi at different concentrations:
  - *Aspergillus ochraceus*, *A. carbonarius*, *A. niger*.
- Direct extractions on spores

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


Parche
Parch
Café vert
Green Coffee
Café parche
Parchment coffee
Jus mulcilage
Mucilage water
Café démucilaginé
Demucilaginated coffee
Café dépulpé
Depulped Coffee
Cerise
Cherry


Parch
Green Coffee
Parchment coffee
Washed Coffee
Cleaning Water
Fermented Coffee
Depulped Coffee
Cherry
Green Coffee
Parchment coffee
Washed Coffee
Cleaning Water
Fermented Coffee
Depulped Coffee
Cherry