International Journal of Food Microbiology 297 (2019) 1–10

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

# Genetic diversity and population structure of *Saccharomyces cerevisiae* strains isolated from traditional alcoholic beverages of Côte d'Ivoire

Charles Y. Tra Bi[a,b,*,1], Tiemele L.S. Amoikon[a,b,1], Clémentine A. Kouakou[a], Jacques Noemie[b,2], Marine Lucas[b], Cécile Grondin[b], Jean-Luc Legras[c], Florent K. N'guessan[a], Theodore N. Djeni[a], Marcellin K. Djè[a], Serge Casaregola[b]

[a] *Laboratoire de Biotechnologie et Microbiologie des Aliments, Unité de Formation et de Recherche en Sciences et Technologie des Aliments (UFR-STA), Université Nangui-Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire*

[b] *Micalis Institute, INRA, AgroParisTech, CIRM-Levures, Université Paris-Saclay, 78350 Jouy-en-Josas, France*

[c] *SPO, Université Montpellier INRA, Montpellier SupAgro, F-34060 Montpellier, France*

ARTICLE INFO

*Keywords:*
Microsatellite
Population
*Saccharomyces cerevisiae*
Diversity
Traditional beverage
Genotyping

ABSTRACT

In order to assess the genetic diversity and population structure of indigenous *S. cerevisiae* from Côte d'Ivoire, a total of 170 strains were isolated from four traditional alcoholic beverages through nine regions. Microsatellite analysis performed at 12 loci revealed that strains of palm oil and raffia wine were genetically related, unlike those of tchapalo and ron wine which formed two s from palm oil wine and raffia wine were clearly inbred. In comparison with the European, North American, Asian and others West African populations, Ivorian population was well defined, although most of these strains were admixed. Among these strains, only isolates from raffia wine appeared to have alleles in common to all populations.

## 1. Introduction

*Saccharomyces cerevisiae* is one of the main actor involved in various biotechnology processes. These last decades, several studies have been focused on genetic diversity and biogeographical distribution of diverse *S. cerevisiae* strains. Molecular techniques used for this purpose among many others are pulsed field gel electrophoresis (PFGE) (Vezinhet et al., 1990), restriction analysis of the mitochondrial DNA (mtDNA-RFLP) (Aigle et al., 1984; Querol et al., 1992), amplified fragment length polymorphism (AFLP) (Azumi and Goto-Yamamoto, 2001), amplification of interdelta regions by PCR (Ness et al., 1993; Legras and Karst, 2003) and microsatellite markers (Techera et al., 2001; Legras et al., 2005). Microsatellite analyses have offered significant advances in understanding distribution and dynamics of *S. cerevisiae* population from different terroirs. Thus, Viel et al. (2017) have detected specificities of *S. cerevisiae* at vineyard scale from the North-East of Italy, suggesting a geographic differentiation according to the winemaking region with a widespread dissemination of *S. cerevisiae* industrial strains that was very high in the areas where the native strains abundance was low. Likewise, microsatellite analyses on *S. cerevisiae* populations isolated from African traditional fermented foods allowed to highlight the presence of an African phylogenetic branch separate from European (bread, wine, beer, cheese) and Asian strains (sake) (Legras et al., 2007; Ezeronye and Legras, 2009; Tapsoba et al., 2015), which was confirmed with recent genomic data (Liti et al., 2009; Peter et al., 2018). This suggests that African traditional fermented foods could be a reservoir of genetically different strains of *S. cerevisiae* to be explored for applications such as food, bioethanol production, biopreservation, biocontrol, etc. Most of *S. cerevisiae* commonly used in biotechnology have been isolated from natural niches and have undergone various domestication events that have resulted in the selection of strains adapted to human activities (technological strains, etc.). Although several studies have been performed on *S. cerevisiae* isolated from some natural niches (Naumov et al., 1998; Sniegowski et al., 2002; Lorca et al., 2018), it seems important to assess the structure, the genetic diversity and the distribution of native *S. cerevisiae* obtained from African, and Ivoirian niches particularly, which has been very little studied so far. The characterization of these unexplored ecological niches may provide

---

[*] Corresponding author at: Laboratoire de Biotechnologie et Microbiologie des Aliments, Unité de Formation et de Recherche en Sciences et Technologie des Aliments (UFR-STA), Université Nangui-Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire.

*E-mail address:* charlestrabiyouan@yahoo.fr (C.Y. Tra Bi).

[1] These authors have contributed equally to this work.

[2] Present address: Bioger, INRA/AgroParisTech, 78850, Thiverval-Grignon, France.

https://doi.org/10.1016/j.ijfoodmicro.2019.03.001
Received 17 September 2018; Received in revised form 25 February 2019; Accepted 1 March 2019

new genotypes with beneficial properties for biotechnological applications. The aim of this work was firstly to assess the genetic diversity of *S. cerevisiae* strains present in traditional beverages from Côte d'Ivoire compared to other African beverages and then characterize the population structure of the isolates of this species. For this purpose, this study focused on the yeast populations isolated from four fermented beverages obtained with different vegetal material. These beverages were tchapalo beer produced from sorghum (*Sorghum bicolor*) and palm oil, raffia and ron wines respectively obtained from *Elaeis guineensis*, *Raphia hookeri* and *Borassus aethiopum* sap fermentation.

## 2. Material and methods

### 2.1. Sample collection and yeast isolation

Samples of palm wine (raffia, palm oil and ron wines) were collected during ten sampling campaigns in different regions of Côte d'Ivoire. Sampling areas for raffia wine were located at Grand-Lahou, Alepé, Adzopé and Abengourou; those for palm oil wine were located at Bingerville, Bonoua, Grand-Lahou, Alépé and Attinguié while those for ron wine were located at Toumodi. Sorghum beer or tchapalo samples were collected during four sampling campaigns at Abidjan and Bingerville. These sampling areas were located in south-eastern and middle of Côte d'Ivoire as indicated by the sample map (Fig. 1). Distance between the sampling areas and the city of Abidjan as well as their geographical coordinates are shown in the Table 1. Local producers in 26 different sites were contacted and sampling was carried out from September 2014 to January 2015 and from August 2016 to September 2016. Sampling was twice a day (morning and evening) done at weekly intervals, to collect samples of 9 to 15 h fermentation duration. Samples were collected into pre-sterilized 250 mL Plexiglas containers, and immediately immersed in an isothermal box containing dry ice, and brought to the laboratory within 3 h.

Total titratable acidity (TTA), pH, and total soluble sugars (TSS) for each sample were determined as previously described (Tra Bi et al., 2016). For yeast isolation, each beverage sample (1 mL) was directly diluted in tenfold series in buffered peptoned water (BIO-RAD, France) and aliquots (0.1 mL) were plated in duplicate on Yeast Extract Peptone Dextrose (YPD) agar. After incubation at 30 °C for 3–5 days, ten yeast colonies were randomly selected from each sample and purified by streaking two times on YPD agar. *Saccharomyces* yeasts were presumptively isolated by cultivation of purified colonies on lysine agar medium (Sigma-Aldrich, France). Thus, a total of 262 isolates were selected for molecular identification and maintained at −80 °C in YPD broth containing 20% (v/v) glycerol (Merck, France).

Variance analysis (ANOVA) and Tukey HSD tests (Honestly Significant Difference) with R statistical software v3.4.1 (R Core Team, 2017) were used to compare the physicochemical and microbiological characteristics of each beverage taken from the different sampling areas. Differences were considered significant for values of $P < 0.05$.

### 2.2. Molecular identification of Saccharomyces species

The species level identity of *Saccharomyces* strains was checked initially by non-transcribed spacer PCR-Restriction Fragment Length Polymorphism (NTS2 PCR-RFLP) analysis using *Alu*I and *Ban*I enzymes in comparison with reference strains. From strains showing *S. cerevisiae* NTS2 PCR-RFLP profile (Nguyen and Gaillardin, 1997), thirty representative strains were selected for D1/D2 region sequencing (Kurtzman and Robnett, 1998). The D1/D2 region of 26S rRNA gene was amplified with the primer pair NL-1 (5-GCATATCAATAAGCGGAGGAAAAG-3) and NL-4 (5-GGTCCGTGTTTCAAGACGG-3) (Kurtzman and Robnett, 1998). Reactions were performed in an automatic thermal cycler (Gene Amp PCR System 2700, Thermo Fisher Scientific) under the following conditions: initial denaturation at 94 °C for 4 min followed by 30 cycles of denaturation at 94 °C for 30 s, primer annealing at 54 °C for 40 s and DNA extension at 72 °C for 1 min 30 s. A final extension was completed at 72 °C for 7 min. Amplicons were visualized on 0.8% agarose gel. Finally, 20 μL of PCR products were sequenced on both strands with the primers used for the PCR amplification (Eurofins, Germany). Assembly of both strands sequences was performed using the phred/phrap/consed suite (Gordon et al., 1998). Identification queries were fulfilled by a BLAST search of the National Center for Biotechnology Information database (NCBI, Bethesda, USA) and the YeastIP databases (http://genome.jouy.inra.fr/yeastip/).

### 2.3. Microsatellite analysis

The genomic DNA extraction of isolates was performed following the procedure described by Hoffman and Winston (1987), based on a

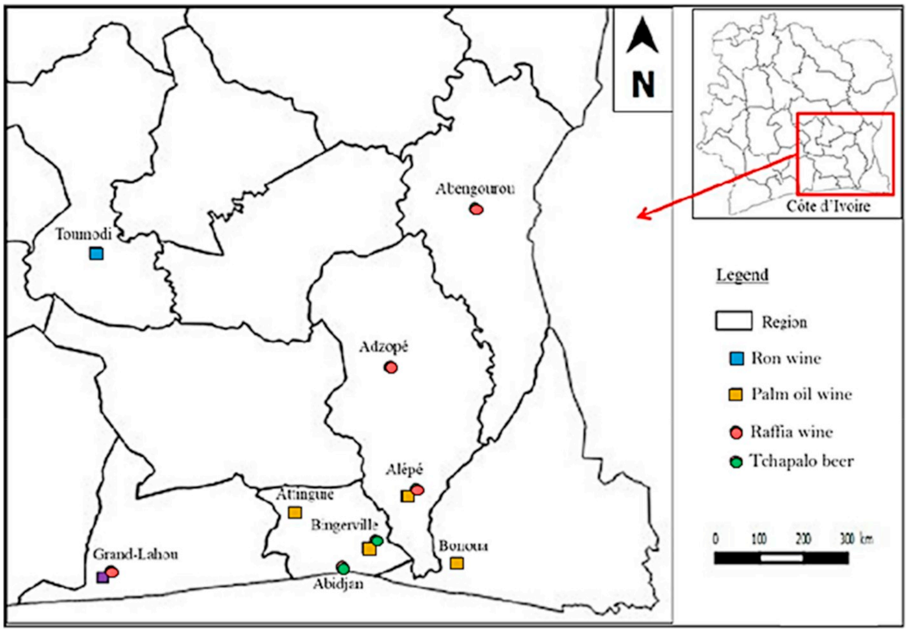


**Fig. 1.** Map of Côte d'Ivoire showing the areas from where samples were collected.

**Table 1**
Geographical information of the sampling areas and sample codes.

| Sampling areas | Geographical coordinates | Distance between the sampling area and the city of Abidjan (Km) | Beverage collected | Code |
|---|---|---|---|---|
| Abidjan | l: 5°18.5796′ N<br>L: 4°0.7596′ W | 0 | Tchapalo beer | TLM/Ab/ TT14/TPA/ |
| Bingerville | l: 5°21.3486′ N<br>L: 3°53.1222′ W | 18 | Palm oil wine<br>Tchapalo beer | BingerVP<br>Binger-T |
| Attinguié | l: 5°29′40″ N<br>L: 4°03′06″ W | 22 | Palm oil wine | AttgiéP-Vp |
| Alépé | l: 5°29′46″ N<br>L: 3°39′49″ W | 45 | Palm oil wine<br>Raffia wine | PA<br>AR |
| Bonoua | l: 5°16.3482′ N<br>L: 3°35.775′ W | 59 | Palm oil wine | Bon Vp |
| Adzopé | l: 6°06′24″ N<br>L: 3°51′42″ W | 104 | Raffia wine | Adz-R |
| Grand-Lahou | l: 5° 8′ 7 N<br>L: 5° 1′ 26s′ W | 152 | Palm oil wine<br>Raffia wine | PG<br>GR |
| Toumodi | l: 6°33.4794′ N<br>L: 5°1.0614′ W | 198 | Ron wine | TR |
| Abengourou | l: 6°43′46″ N<br>L: 3°29′47″ W | 210 | Raffia wine | Abeng R |

**Table 2**
Physico-chemical characteristics and yeast count of alcoholic beverages of the study.

| Beverages | | pH | Total titratable acidity (%) | Total soluble sugars (°Brix) | Yeast count (CFU/mL) |
|---|---|---|---|---|---|
| Palm oil wine | Average | $3.73 \pm 0.09^{*}$ | $0.80 \pm 0.05^{*}$ | $5.41 \pm 0.77^{*}$ | $1.4 \pm 0.1 \times 10^{7*}$ |
| | Minimum | 3.45 ± 0.04 | 0.75 ± 0.07 | 3.72 ± 1.13 | $9.7 \pm 0.5 \times 10^{6}$ |
| | Maximum | 4.30 ± 0.19 | 0.87 ± 0.03 | 6.65 ± 1.41 | $1.7 \pm 0.2 \times 10^{7}$ |
| Raffia wine | Average | $3.80 \pm 0.12^{*}$ | $0.81 \pm 0.10^{*}$ | $4.35 \pm 0.79^{\dagger}$ | $1.3 \pm 0.2 \times 10^{7*}$ |
| | Minimum | 3.21 ± 0.26 | 0.47 ± 0.04 | 3.00 ± 0.30 | $9.5 \pm 2.3 \times 10^{6}$ |
| | Maximum | 4.35 ± 0.01 | 1.35 ± 0.07 | 7.00 ± 0.40 | $4.8 \pm 1.8 \times 10^{7}$ |
| Ron wine | Average | $3.72 \pm 0.12^{*}$ | $0.68 \pm 0.09^{\dagger}$ | $4.88 \pm 0.40^{\dagger}$ | $1.1 \pm 0.3 \times 10^{8\dagger}$ |
| | Minimum | 3.54 ± 0.08 | 0.48 ± 0.11 | 4.03 ± 0.25 | $1.9 \pm 0.7 \times 10^{7}$ |
| | Maximum | 3.91 ± 0.21 | 1.00 ± 0.14 | 5.80 ± 0.30 | $2.6 \pm 0.6 \times 10^{8}$ |
| Tchapalo beer | Average | $3.46 \pm 0.13^{*}$ | $0.93 \pm 0.03^{\ddagger}$ | $7.70 \pm 1.04^{\ddagger}$ | $1.7 \pm 0.1 \times 10^{7*}$ |
| | Minimum | 3.38 ± 0.1 | 0.92 ± 0.02 | 5.81 ± 0.57 | $1.2 \pm 0.1 \times 10^{7}$ |
| | Maximum | 3.5 ± 0.14 | 0.94 ± 0.04 | 10.87 ± 2.0 | $2.4 \pm 0.1 \times 10^{7}$ |

The values expressed are the average of three measurements. On the same column, mean values with the same symbol are not significantly different (Tukey HSD test range at $P < 0.05$).

mechanical grinding of cells in presence of detergent, followed by several steps of protein and RNA removal from a 24 h yeast culture in YPD broth. Microsatellite loci analysis was performed according to Legras et al. (2007). Amplification was performed at 12 loci combined in two multiplex of 6 loci (Table S1 in the supplemental material), and the size of fluorescent amplicons was measured on an ABI3100 (Applied Biosystems) capillary electrophoresis device, using Gene Scan HD400Rox size standard (Applied Biosystems). These genotypes were compared to data obtained in previous work on strains from other origins.

### 2.4. Population analysis

The evaluation of relationships between *S. cerevisiae* strains was carried out from the genetic relative dissimilarity distance which reflects the number of alleles differing between two individuals, and calculated with the library POPPR (v2.5.0) under R statistical software v3.4.1 (R Core Team, 2017). Trees were obtained from distance matrices with R library ape v5.0, and drawn using R library ggtree v1.10.5 (Yu et al., 2017). In order to assess genetic structure in *S. cerevisiae* populations, two different approaches were used. The first one was Bayesian clustering analysis implemented with InStruct 2.3.4 software (Gao et al., 2007) to identify the most likely number of genetic clusters or 'populations' (denoted by K) and assign individuals to their most likely cluster. In order to deduce ancestry on that set of strains, 8 runs were performed with Instruct for values from K = 2 to 17, and 8 additional runs for values from K = 14 to 16. The most likely K value was identified as the value giving the lowest mean value of Deviance Information Criterion (DIC) of the different runs. The results of the 16 runs of the best value of K were combined with CLUMPAK (Kopelman et al., 2015) according to the LargeK Greedy method. The second was a multivariate method based on discriminant analysis in principal components (DAPC) (Jombart et al., 2010) and implemented with the R Adegenet package v2.1.0 (Jombart, 2008) in R v3.4.1 (R Core Team, 2017). DAPC identified clusters of genetically related organisms by partitioning genetic variability into clusters that maximize between-group and minimize within-group differentiation. This approach was an ideal clustering algorithm for datasets that does not followed Hardy–Weinberg equilibrium. Analysis was performed taking into account alpha score function to choose the optimal number of principal components for the analysis of dataset (López-Uribe et al., 2016) due to the changes of the individual membership probability according to the number of PCA axes retained. Population analyses were performed using the hierfstat v0.04-22 R package (Goudet, 2005) for Nei (1973) estimates of effect of subpopulations compared to the total population (Fst). Population differentiation according to sampling area was tested with OBSTRUCT (Gayevskiy et al., 2014). The inbreeding coefficient of an individual relative to the subpopulation (Fis) was estimated with hierfstat. Average selfing rates were estimated as 1 – o, where o the outcrossing rate is o = (1 – Fis) / (1 + Fis).

**Table 3a**

Pairwise Fst distance matrix between populations isolated from different beverages. Fst is calculated according to Nei (1973). Fst values are given in the lower matrix and upper matrix indicates *P*-value estimated from 1000 permutations of the genotypes dataset.

| | Palm oil wine | Raffia wine | Ron wine | Tchapalo beer |
|---|---|---|---|---|
| Palm oil wine | 0.000 | 0.008 | 0.001 | 0.001 |
| Raffia wine | 0.021 | 0.000 | 0.001 | 0.001 |
| Ron wine | 0.061 | 0.069 | 0.000 | 0.001 |
| Tchapalo beer | 0.107 | 0.074 | 0.073 | 0.000 |

**Table 3b**

Pairwise Fst distance matrix between sampling areas. Fst is calculated according to Nei (1973). Fst values are given in the lower matrix and upper matrix indicates *P*-value estimated from 1000 permutations of the genotypes dataset.

Palm oil wine

| | Attingué | Grand-Lahou | Alépé | Bingerville | Bonoua |
|---|---|---|---|---|---|
| Number of individuals | 6 | 7 | 7 | 5 | 4 |
| Attingué | 0.000 | 0.022 | 0.035 | 0.077 | 0.002 |
| Grand-Lahou | 0.066 | 0.000 | 0.001 | 0.033 | 0.001 |
| Alépé | 0.045 | 0.070 | 0.000 | 0.008 | 0.001 |
| Bingerville | 0.093 | 0.092 | 0.084 | 0.000 | 0.001 |
| Bonoua | 0.070 | 0.064 | 0.073 | 0.118 | 0.000 |

Raffia wine

| | Grand-Lahou | Alépé | Adzopé | Abengourou |
|---|---|---|---|---|
| Number of individuals | 16 | 35 | 6 | 7 |
| Grand-Lahou | 0.000 | 0.003 | 0.008 | 0.044 |
| Alépé | 0.011 | 0.000 | 0.019 | 0.031 |
| Adzopé | 0.049 | 0.022 | 0.000 | 0.051 |
| Abengourou | 0.054 | 0.026 | 0.061 | 0.000 |

Tchapalo beer

| | Abidjan | Bingerville |
|---|---|---|
| Number of individuals | 17 | 2 |
| Abidjan | 0.000 | 0.198 |
| Bingerville | 0.022 | 0.000 |

Values are significantly different for *P*-value < 0.05.

## 3. Results

### 3.1. Characteristics of beverages

The global characteristics of the four different beverages from which strains were isolated in this study were presented in Table 2. It appeared from this investigation that these four beverages had in general a similar acidic pH and a Total Titratable Acidity (TTA) varying between 0.47 and 1.35. The soluble sugars content varied from one beverage to another with the highest content in tchapalo. The yeast load observed in the Ivorian beverages sampled was generally of the same order. Average values were $1.1 \times 10^8$ CFU/mL in ron wine, $1.7 \times 10^7$ CFU/mL in tchapalo beer, $1.4 \times 10^7$ CFU/mL in palm oil wine and $1.3 \times 10^7$ CFU/mL in raffia wine (Table 2).

### 3.2. Assessment of Saccharomyces cerevisiae diversity

A total of 170 isolates from Côte d'Ivoire traditional beverages (Table S2 in the supplemental material) were identified as belonging to the species *S. cerevisiae* by using molecular methods. Indeed, the RFLP of NTS2 PCR products showed 2 bands for *Alu*I (1500 and 200 pb) and 3 bands for *Ban*I (660, 450 and 220 pb) typical to *S. cerevisiae* strains (Nguyen and Gaillardin, 1997). Then, the sequencing of the D1/D2 domains of the 26S rDNA confirmed the identity of some strains tested as *S. cerevisiae* with a little sequence divergence of 1 to 4 bp for 16 strains and 100% identity for 14 strains (Table S3 in the supplemental material). Sequence divergence of 3 to 4 bp was already observed from *S. cerevisiae* strains isolated from tchapalo (N'guessan et al., 2011). The 170 isolates derived from four ecological niches sampled at 26different sites from 9 regions (south-eastern and middle) of Côte d'Ivoire. Among them, 58 have been isolated from ron wine, 64 from raffia wine, 29 from palm oil wine and 19 from tchapalo beer. These isolates were subjected to a microsatellite analysis which allowed to generate genotypic data. In order to compare the populations between the different type of beverages, Fst statistics between isolates from tchapalo beer, palm oil, raffia and ron wines were calculated with these data. Population comparisons indicated significant differences between all populations ($P < 0.01$). Fst data showed weak values ranging from 0 to 0.107 (Table 3a), indicating a little genetic differentiation between the significantly different beverage isolates. The smallest differentiation was observed between palm oil wine and raffia wine strains, and in contrast, tchapalo was the most differentiated population from other populations. Ron wine population presented a moderate differentiation from tchapalo and raffia wine.

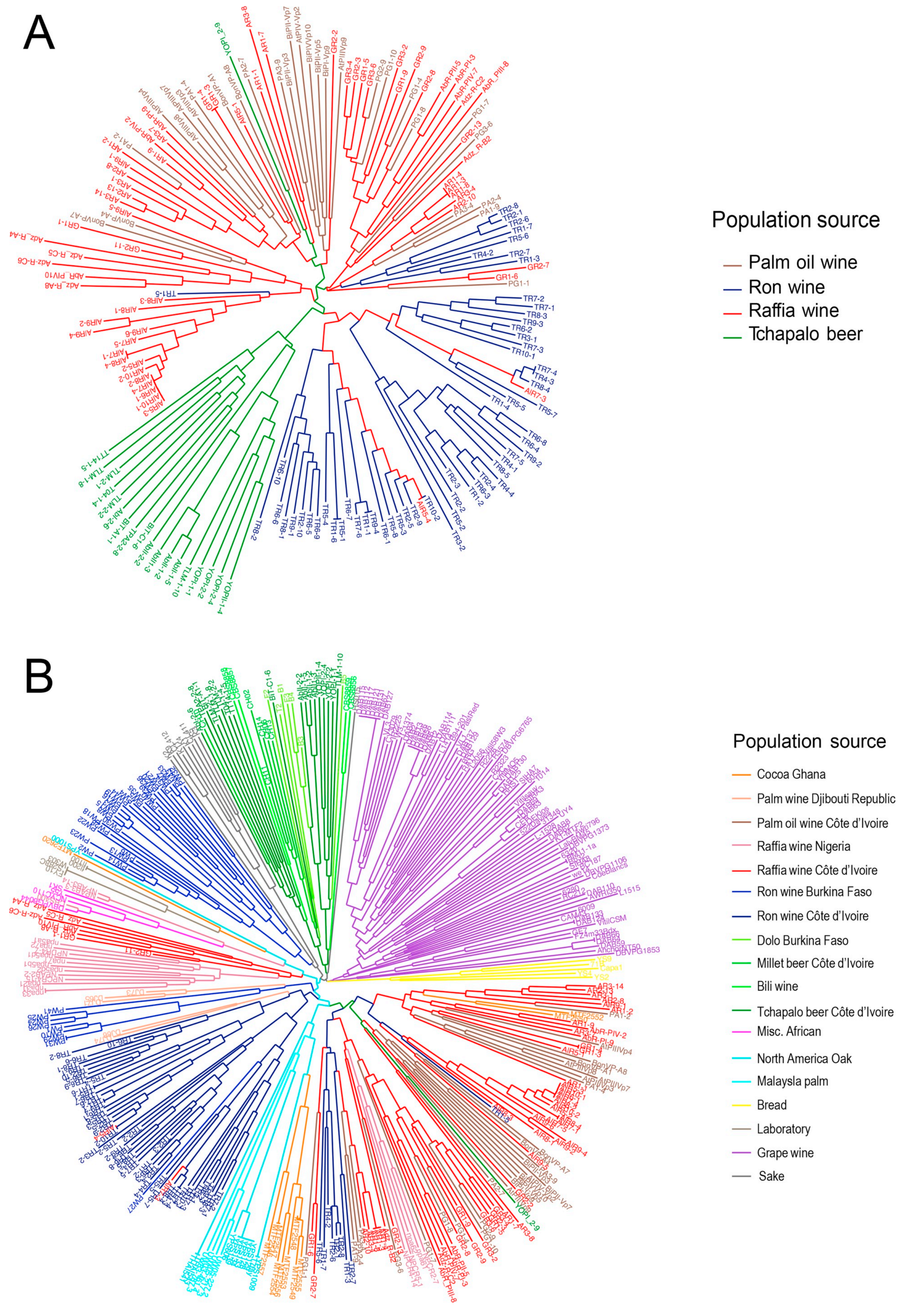
Impact of geographical origin on *S. cerevisiae* diversity was studied for isolates from palm oil wine, raffia wine and tchapalo beer. Ron wine with only one sampling area was not considered for this study. The Nei pairwise Fst distances matrix for the two sampling areas of tchapalo beer (Abidjan and Bingerville) showed low Fst values and non-significant differentiation. In contrast the four sampling areas of raffia wine (Grand-Lahou, Alépé, Adzopé and Abengourou) and most of the five sampling areas of palm oil wine showed low Fst values and significant differentiation between sites (Table 3b). Furthermore, pairwise Fst statistic values for all sampling areas demonstrated no correlation to geographical distance, suggesting that diversity of *S. cerevisiae* was not related to geographical origin. Although isolates of palm oil and raffia wines were genetically related, *S. cerevisiae* isolates from Côte d'Ivoire traditional beverages appeared to be differentiated according to the ecological niche.

The Fis values evaluated for each population were 0.603 and 0.544, respectively, for individuals from palm oil wine and raffia wine, 0.234 and 0.138 for individuals from ron wine and tchapalo beer that contained strains not corresponding to one specific fermentation (Table 4). Such high values of Fis which indicate a low outcrossing rate (and a high mean inbreeding rate) as well as the high observed homozygosity of strains isolated from palm oil wine and raffia wine (26 and 25% of

**Table 4**

Fis value per population and corresponding estimate of selfing rate.

| | Ecological niche | | | |
|---|---|---|---|---|
| | Raffia wine | Palm oil wine | Tchapalo beer | Ron wine |
| Number of individuals | 64 | 29 | 19 | 58 |
| Fis | 0.603 | 0.544 | 0.138 | 0.214 |
| Outcrossing estimate ($o = (1 - Fis) / (1 + Fis)$) | 0.25 | 0.30 | 0.76 | 0.65 |
| Selfing estimate ($= 1 - o = 2Fis / (1 + Fis)$) | 0.75 | 0.70 | 0.24 | 0.35 |



(caption on next page)

**Fig. 2.** **a**: Phylogenetic tree among Ivorian *S. cerevisiae* strains. The tree is drawn according to neighbour-joining method from a pairwise individual distance matrix calculated with relative dissimilarity distance between 170 strains and based on the polymorphism at 12 loci.
**b**: Phylogenetic tree among *S. cerevisiae* strains of diverse geographical origins and substrates. The tree is drawn according to the neighbour-joining method from a pairwise individual distance matrix calculated with relative dissimilarity distance between the 284 strains (from 18 different ecological niches) and based on the polymorphism at 12 loci. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

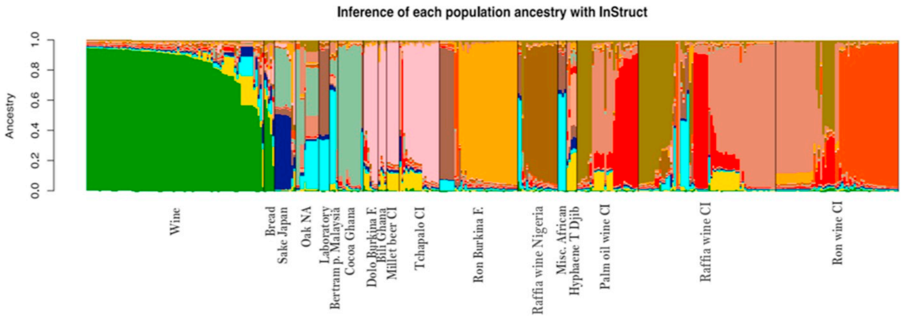


**Fig. 3.** Inferred population structure using InStruct program on the 384 *S. cerevisiae* strains. Deviance information criterion indicated the most likely population number to be K = 15. *S. cerevisiae* populations are delimited vertically according to their ecological origin. Colours represent the different alleles that constitute each population.

observed sites are heterozygotes), suggest that palm oil wine and raffia wine are inbred. In contrast, the low Fis values which indicate a high outcrossing rate (and a low mean selfing rate) as well as the high observed heterozygosity (52 and 76% of observed sites are heterozygotes) for isolates from ron wine and tchapalo beer suggest more outbred populations.

### *3.3. Genetic relationship among strains*

The phylogenetic relationships among Ivorian strains of *S. cerevisiae* were highlighted by a phylogenetic tree (Fig. 2a). This dendrogram showed that strains of tchapalo (in green) and ron wine (in blue) formed two distinct and homogeneous clusters, unlike those of palm oil (in brown) and raffia wines (in red). Strains of the latter two groups appeared mixed in the remaining clusters.

To further detect the specificities of these Ivorian *S. cerevisiae* populations in comparison to populations from other African countries, and in comparison to well characterized populations such as those from wine, sake, and bread, we built a tree including the 170 Ivorian strains and 214 strains from multiple African countries or other origins (Fig. 2b) analyzed in a previous work (Tapsoba et al., 2015). This consensus neighbour-joining tree was obtained from a pairwise individual distance matrix calculated with relative dissimilarity distance. It showed several clusters of different genetic origin closely related to geographic origin. Thus, there was a cluster formed from European strains: wine and bread strains. Five clusters of strains originated from African substrates, one of which consists of tchapalo and millet beer (different shades of green) and the four others of different types of palm wine. The last two clusters encompassed Asian and North American strains. Ivorian strains are mainly encountered as sub groups inside the main clusters. In contrast to sorghum beer strains, ron wine strains from Côte d'Ivoire are found in two main clusters, one close to Burkina Faso ron wine strains, and in a second different cluster. A similar situation can be observed for raffia wine strains: one group of strains is detected close to Nigerian raffia wine strains, whereas the second group is different.

### *3.4. Population structure*

Population structure was inferred with the Bayesian clustering software InStruct which does not assume Hardy-Weinberg equilibrium, but instead assigns individuals proportional membership coefficients in different populations based on inbreeding rates, which is well suited for *S. cerevisiae* population analysis (Gao et al., 2007; Martiniuk et al., 2016). Deviance information criterion indicated the most likely ancestral population number to be K = 15.

Each population was labelled by the geographic location and/or substrate from which the strains were isolated (Fig. 3). The 15 populations included 11 African beverage populations among them, five originated from Côte d'Ivoire, two from European wine and bread populations, one from the Asian sake population and one from the North American oak population. The InStruct inferences revealed that most strains were assigned to a single ancestry cluster although admixtures were observed. About 8 out of 18 clusters were composed of individuals with one ancestral cluster membership coefficients higher than 0.80. The 10 other clusters (bread, sake from Japan, oak from North-America, laboratory, Bertram palm from Malaysia, various origins from Africa, *Hyphaene thebaica* from Djibouti, raffia, palm oil and ron wines from Côte d'Ivoire) appeared as admixtures. A small number of individuals within ron (*Borassus aethiopum*) wine strains had alleles common with those of ron strains from Burkina Faso, raffia and palm oil wines from Côte d'Ivoire. Strains of palm oil wine have some alleles in common with strains from Djibouti, Ghana (bili), Burkina Faso (dolo), other strains from Côte d'Ivoire (tchapalo and millet beer) and European wine strains. Strains of raffia wine appeared to have alleles in common with all populations, but in small proportion (Table S2 in the supplemental material).

In order to improve the detection of population structure of this study, a discriminant principal component analysis (Jombart et al., 2010) based on 80% of the global variation obtained from the 50 first PCA axis was conducted (Fig. 4). The first two axes enabled differentiation of sorghum and millet beers strains (different shades of pink in the lower part of Fig. 4a) from Burkina Faso ron wines strains (shade of brown), Côte d'Ivoire (coral) and miscellaneous African strains (magenta) in the upper part of Fig. 4a. Genetic differentiation between African strains following some of their ecological niche was observed in the second axis: strains from the sap of *Arecaceae* family plants of the (palm oil palm, raffia palm, *Borassus* palm…) in the upper quadrant of Fig. 4a were distinguished from those *Poaceae* family (millet and sorghum) in the lower part. The cluster strains corresponding to Ivorian tchapalo beer isolates were colocalized with Burkina Faso dolo beer isolates and Côte d'Ivoire millet beer strains. As for axes 3 and 4 (Fig. 4b), they highlighted the differentiation between North-America populations and Ghana cocoa strains from that of the rest of the world, and the axis 4 differentiate Nigeria palm strains (and to a lower extent sake strains) from other strains. The two plots (Fig. 4) showed that palm oil, raffia and ron wines from Côte d'Ivoire were also clustered but these clusters overlapped with clusters strains from Djibouti palm, Nigeria raffia wine, Burkina Faso ron wine and African miscellaneous origins. This clustering was also in agreement with the clustering obtained at K = 15 for InStruct.

Obstruct tests were performed to evaluate if the population

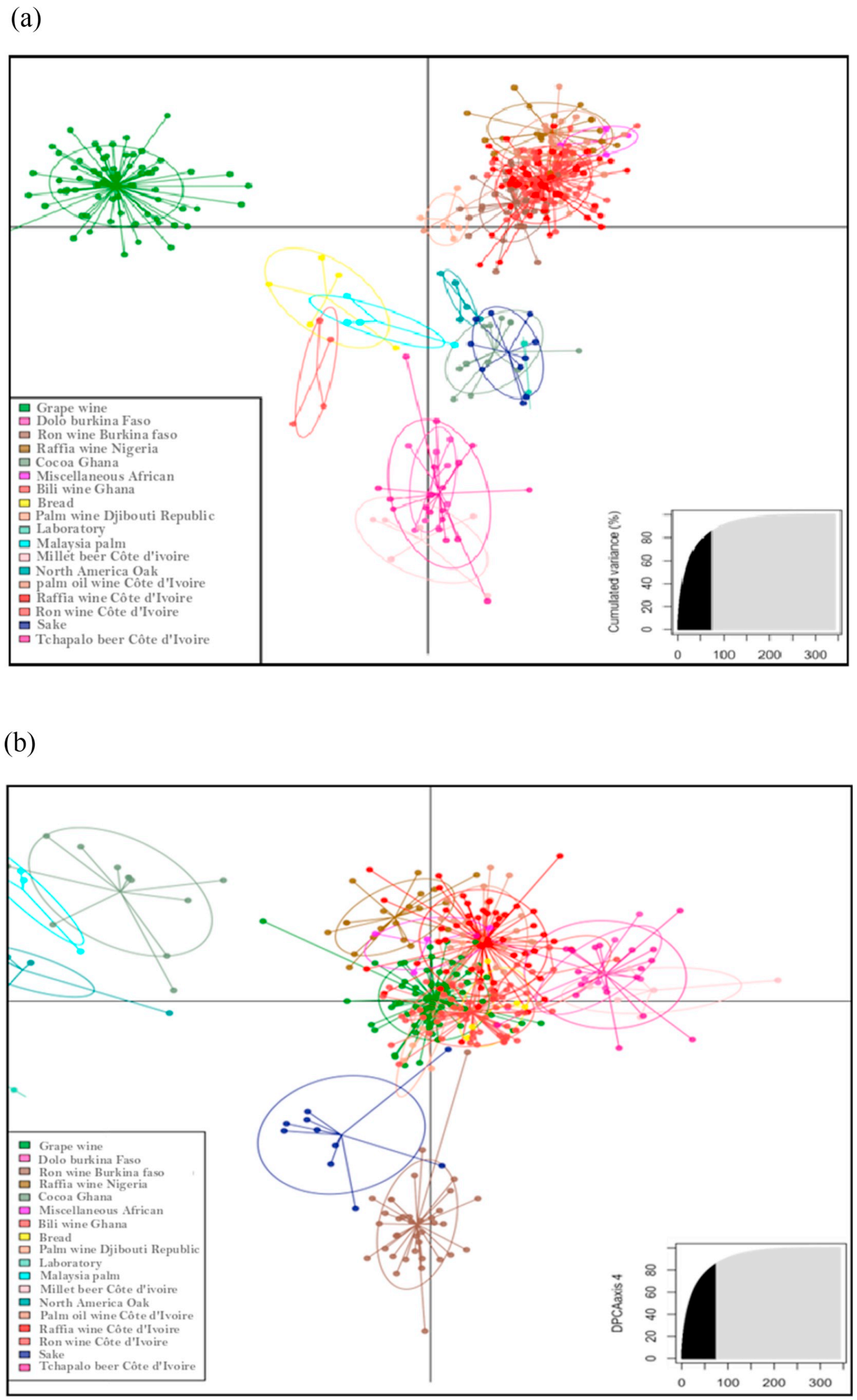


**Fig. 4.** Discriminant principal component analysis (DPCA) (a): plot showing representation of the different clusters according to axes 1 and 2; (b) plot showing representation of the different clusters according to axes 3 and 4. Clusters are shown by different colours and inertia ellipses, while dots represent individuals.

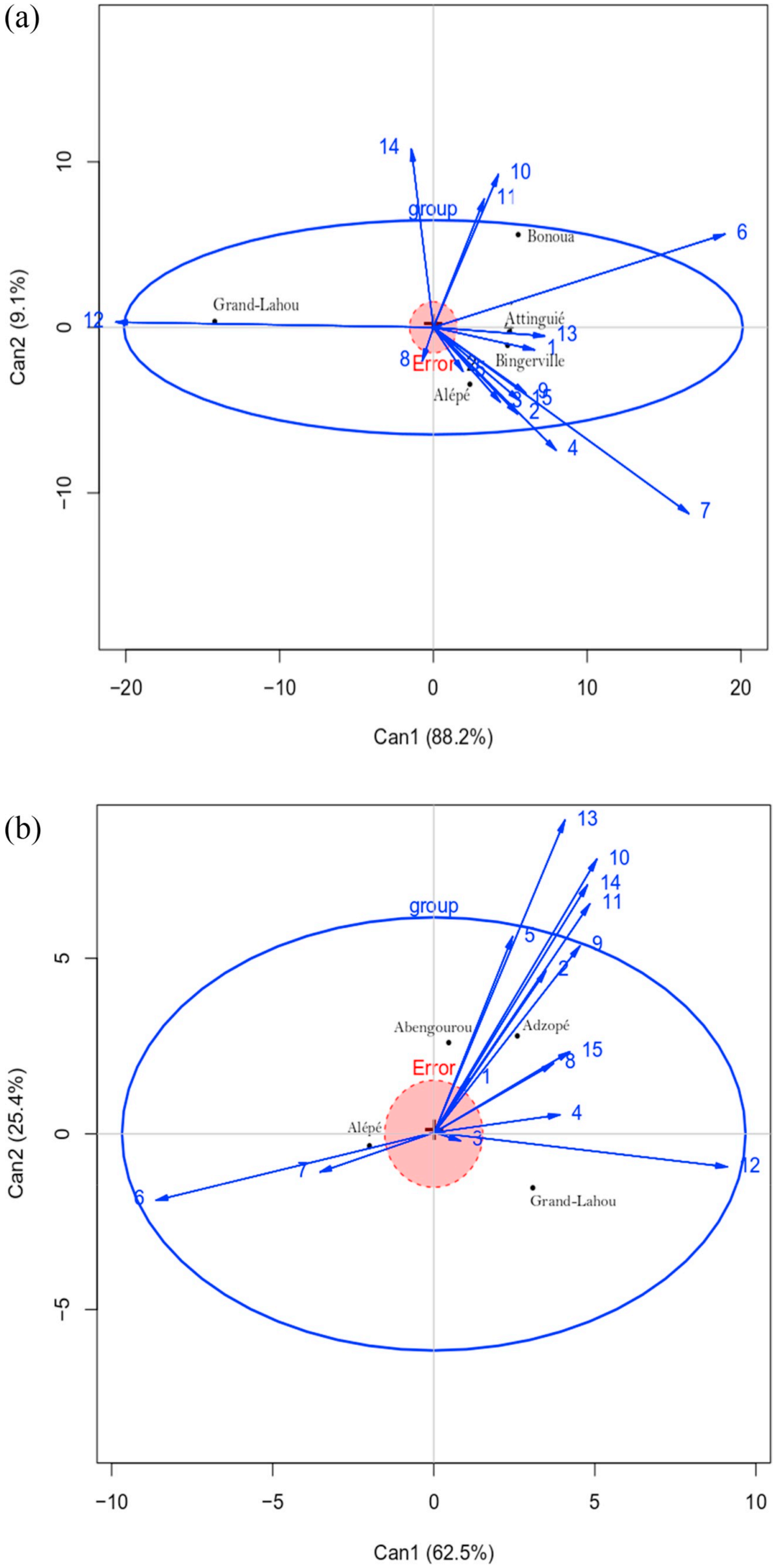


**Fig. 5.** Canonical discriminant analysis of the ancestry obtained for palm oil wine (a) and raffia wine (b) isolates from Côte d'Ivoire with OBSTRUCT, after removal of strains from other countries. The outer blue ellipsoid labelled group reflects the variation of the group means around the grand mean while the red circle reflects the pooled within-group dispersion and covariation. The black points indicate predefined populations while numbers at the arrows indicate inferred populations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

structure revealed in Bayesian ancestry profiles obtained from Instruct was correlated with the geography and confirmed of Nei pairwise Fst distance. For this analyse, only palm oil and raffia wines isolates, which had more than two sampling areas were considered. Obstruct analysis and canonical discriminant analysis clearly showed that the differences between isolates for most sampling areas of palm oil wine (Attinguié, Grand-Lahou, Alépé, Bingerville and Bonoua) were significant (Fig. 5, Table S4a in the supplemental material). Strains isolated from Bonoua and Grand-Lahou were very different from the others. For strains from sampling areas of raffia wine, the differences between populations were also significant with the isolates from Alépé and Grand-Lahou and very different from the others isolates (Fig. 5, Table S4b in the supplemental material).

## 4. Discussion

This study was centered on the genetic diversity and population structure of indigenous *S. cerevisiae* from Cote d'Ivoire, as well as the factors that may explain the structure of the resulting populations. For this purpose, four traditional alcoholic beverages (raffia wine, palm oil wine, ron wine and tchapalo beer) collected in nine regions of Côte d'Ivoire were studied.

The pH of these beverages, which fluctuated between 3 and 5, were similar to those reported by several authors in palm wines after the first day of tapping (Amoa-awua et al., 2007; Karamoko et al., 2012) and in tchapalo beer produced by traditional brewers (Aka et al., 2008). These ranges of pH could be a major advantage for the bio-preservation of these beverages by protecting them from the development of some pathogenic flora (Aka et al., 2008; Yao et al., 2009). The Total Titratable Acidity (TTA) and Total Soluble Sugars (TSS) contents were variable from one beverage to another, while the yeast average load was around $10^7$ to $10^8$ CFU/mL in the four beverages. The low TSS contents observed in beverages of this study could be related to the high yeast load, but also to the rest of the microbiota of these beverages. TTA rates observed in beverages could be due to the production of a variety of organic acids by yeasts and bacteria (N'guessan et al., 2008; Karamoko et al., 2012). Yeast loads found in this study could probably be due to the sum of various physical, chemical, and biotic factors such as temperature, humidity, presence of nutrients, such as sugars (Santiago-Urbina et al., 2015). Similar yeast loads were reported in traditional fermented beverages produced in Africa (Aka et al., 2008; Stringini et al., 2009; Karamoko et al., 2012). Diversity analysis indicated that ecological niche had a major influence on *S. cerevisiae* population's native of Côte d'Ivoire. Fis values of palm oil and raffia wines strains (0.603 and 0.544) revealed an excess of homozygote individuals which is very likely due to high inbreeding within each population rather than to population differentiation (Albertin et al., 2014). Schuller and Casal (2007) also observed a significant excess of homozygotes in their study on three vineyards located in the northwest of Portugal. These authors explained this fact by asexual reproduction with some cycles of homothallic self-mating (genome renewal). Moreover, these authors proposed as an alternative explanation that a mitotic recombination or gene conversion during asexual reproduction may generate such a low of heterozygosity. The high degree of homozygosity would point to the existence of genetically isolated clonal subpopulations of *S. cerevisiae* strains with distinct genetic constitution. However, the microsatellite loci analyzed here represent only a tiny fraction of the genome and provide an indication of the mean heterozygosity. The heterozygosity detected at these microsatellite loci was found in good agreement with genomic data (i.e. Legras et al., 2007, 2018) but other loci in the genome including those with technological relevance can present at different heterozygosity status.

Evaluation of genetic diversity of Ivorian strains only revealed that isolates sharing the same micro-environment would be genetically similar to each other, regardless of their geographical origin, implying that ecological origin is the main factor responsible for the close phylogenetic relationship between *S. cerevisiae* strains. Impact of geographical origin on genetic diversity evaluated with Obstruct showed a difference between most of strains population according to sampling area, which was in accordance with Nei Fst pairwise distance. However these genetic distances remained low. Phylogenetic tree, Instruct and Discriminant Analysis of Principal Components (DAPC), considering only African strains, presented that subgroups appeared to be formed by differentiation according to ecological origin. One of the possible explanations is that at a relative close geographical distance, insects like bees, wasps, and fruit flies, as well as birds, which are known to be vectors for yeasts, could have homogenized these yeast populations. Also, Human activities (sharing seasonal staff and equipment during the harvest and fermentation periods) could influence the yeast population structure and promote dispersal (Börlin et al., 2016). Although strains of palm oil wine, raffia wine and ron wine were isolated in Côte d'Ivoire, they have been associated to the same cluster containing the strains of other African countries which derived also of plant sap from the *Arecaceae* family (*Elaeis guineensis*, *Raphia hookeri*, *Borassus aethiopum*, *Borassus akeassii* and *Hyphaene thebaica*). The clustering of strains from different traditional beers, notably tchapalo in Côte d'Ivoire, dolo in Burkina Faso and bili bili in Ghana in the same cluster would reflect the history of the inoculum used. In Côte d'Ivoire, sorghum beer was initially produced by populations living in the North and North-East of the country (Djè et al., 2008; N'guessan et al., 2010). Inocula found in Southern and Eastern Côte d'Ivoire may have been introduced by traditional producers from North and North East of Côte d'Ivoire and those of Burkina Faso, Mali and Ghana. An exception to this distribution of strains according to their geographical origin was the genetic affiliation of strains from Asia (Japanese sake, Malaysian palm wine), and from North America (oak) of some Ivorian strains, a result in agreement with those of Liti et al. (2009). Strains from palm oil, raffia wines and tchapalo beer had common alleles with those of European wine, suggesting that these strains could have a hybrid origin as reported by Ezeronye and Legras (2009). This origin could always be explained by the fact that the European colonization of Africa would have led to the introduction of *S. cerevisiae* in the same way, as cachaça strains in Brazil (Barbosa et al., 2018). This particular ecological situation, where African and European wine or bread species are present in the same environment, may have provided the opportunity for crossbreeding (Legras et al., 2007). Our results confirm that the beverage production areas represent a reservoir of indigenous strains with a particular genotypic profile, selected by interactions between yeasts and their environment, as suggested by Martínez et al. (2007).

The presence of few European wine and bread strains alleles, in low proportions among Ivorian strains would have provided them with some genes of interest, expressing phenotypic characteristics which could be interesting to exploit in fermentation processes. However, a thorough analysis of the biotechnological properties of these strains will be necessary to confirm this idea.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.03.001.

## Acknowledgment

This work was financially supported by the International Foundation for Science, Sweden (E/5560-1) and the National Institute for Agricultural Research (INRA, France). We thank Dr. Didier Montet who helps for the project design, Christelle Louis-Mondésir for expert technical assistance and Tionhonkélé Drissa Soro for design of sampling map.

## References

Aigle, M., Erbs, D., Moll, M., 1984. Some molecular structures in the genome of lager brewing yeasts. J. Am. Soc. Brew. Chem. 42, 1–7. https://doi.org/10.1094/ASBCJ-42-0001.

Aka, S., Djeni, N.T., N'guessan, K.F., Yao, K.C., Djè, K.M., 2008. Variabilité des propriétés physico-chimiques et dénombrement de la flore fermentaire du tchapalo, une bière traditionnelle de sorgho en Côte d'Ivoire. Afr. Sci. 4, 274–286.

Albertin, W., Panfili, A., Miot-Sertier, C., Goulielmakis, A., Delcamp, A., Salin, F., Lonvaud-Funel, A., Curtin, C., Masneuf-Pomarède, I., 2014. Development of microsatellite markers for the rapid and reliable genotyping of Brettanomyces bruxellensis at strain level. Food Microbiol. 42, 188–195. https://doi.org/10.1016/j.fm.2014.03.012.

Amoa-awua, W.K., Sampson, E., Tano-Debrah, K., 2007. Growth of yeasts, lactic and acetic bacteria in palm wine during tapping and fermentation from felled oil palm (Elaeis guineensis) in Ghana. J. Appl. Microbiol. 102, 599–606. https://doi.org/10.1111/j.1365-2672.2006.03074.x.

Azumi, M., Goto-Yamamoto, N., 2001. AFLP analysis of type strains and laboratory and industrial strains of Saccharomyces sensu stricto and its application to phenetic clustering. Yeast 18, 1145–1154. https://doi.org/10.1002/yea.767.

Barbosa, R., Pontes, A., Santos, R.O., Montandon, G.G., de Ponzzes-Gomes, C.M., Morais, P.B,, Gonçalves, P., Rosa, C.A., Sampaio, J.P., 2018. Multiple rounds of artificial selection promote microbe secondary domestication – the case of cachaça yeasts. Genome Biol Evol. evy132, doi:https://doi.org/10.1093/gbe/evy132.

Börlin, M., Venet, P., Claisse, O., Salin, F., Legras, J.-L., Masneuf-Pomarede, I., 2016. Cellar-associated Saccharomyces cerevisiae population structure revealed high level diversity and perennial persistence at sauternes wine estates. Appl. Environ. Microbiol. 82, 2909–2918. https://doi.org/10.1128/AEM.03627-15.

Djè, K.M., N'guessan, K.F., Djeni, N.T., Dadié, A.T., 2008. Biochemical changes during alcoholic fermentation in the production of tchapalo, a traditional sorghum beer. Int. J. Food Eng. 4, 1–15. https://doi.org/10.2202/1556-3758.1408.

Ezeronye, O.U., Legras, J.L., 2009. Genetic analysis of Saccharomyces cerevisiae strains isolated from palm wine in eastern Nigeria. Comparison with other African strains. J. Appl. Microbiol. 106, 1569–1578. https://doi.org/10.1111/j.13652672.2008.04118.x.

Gao, H., Williamson, S., Bustamante, C.D., 2007. A Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. Genetics 176, 1635–1651. https://doi.org/10.1534/genetics.107.072371.

Gayevskiy, V., Klaere, S., Knight, S., Goddard, M.R., 2014. ObStruct: a method to objectively analyse factors driving population structure using Bayesian ancestry profiles. PLoS One 9 (1), e85196. https://doi.org/10.1371/journal.pone.0085196.

Gordon, D., Abajian, C., Green, P., 1998. Consed: a graphical tool for sequence finishing. Genome Res. 8, 195–202.

Goudet, J., 2005. Hierfstat, a package for R to compute and test hierarchical F-statistics. Mol. Ecol. Notes 5, 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x.

Hoffman, C.S., Winston, F., 1987. A ten-minute preparation from yeast efficiently releases autonomous plasmids for transformation of Escherichia coli. Gene 57, 267–272. https://doi.org/10.1016/0378-1119(87)90131-4.

Jombart, T., 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129.

Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genomics 11, 94. https://doi.org/10.1186/1471-2156-11-94.

Karamoko, D., Djeni, N.T., N'guessan, K.F., Bouatenin, K.M.J.-P., Djè, K.M., 2012. The biochemical and microbiological quality of palm wine samples produced at different periods during tapping and changes which occured during their storage. Food Control 26, 504–511. https://doi.org/10.1016/j.foodcont.2012.02.018.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I., 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across. Mol. Ecol. Resour. 15, 1179–1191. https://doi.org/10.1111/17550998.12387.

Kurtzman, C.P., Robnett, C.J., 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek 73, 331–371. https://doi.org/10.1023/A:1001761008817.

Legras, J.-L., Karst, F., 2003. Optimisation of inter-deltas analysis for Saccharomyces cerevisiae strain characterisation. FEMS Microbiol. Lett. 221, 249–255. https://doi.org/10.1016/S0378-1097(03)00205-2.

Legras, J.-L., Ruh, O., Merdinoglu, D., Karst, F., 2005. Selection of hypervariable microsatellite loci for the characterization of Saccharomyces cerevisiae strains. Int. J. Food Microbiol. 102, 73–83. https://doi.org/10.1016/j.ijfoodmicro.2004.12.007.

Legras, J.-L., Merdinoglu, D., Cornuet, J.-M., Karst, F., 2007. Bread, beer and wine: Saccharomyces cerevisiae diversity reflects human history. Mol. Ecol. 16, 2091–2102. https://doi.org/10.1111/j.1365-294X.2007.03266.x.

Legras, J.-L., Galeote, V., Bigey, F., Camarasa, C., Marsit, S., Nidelet, T., Sanchez, I., Couloux, A., Guy, J., Franco-Duarte, R., Marcet-Houben, M., Gabaldon, T., Schuller, D., Sampaio, J.P., Dequin, S., 2018. Adaptation of S. cerevisiae to fermented food environments reveals remarkable genome plasticity and the footprints of domestication. Mol. Biol. Evol. 35, 1712–1727. https://doi.org/10.1093/molbev/msy066.

Liti, G., Carter, D.M., Moses, A.M., Warringer, J., Parts, L., James, S.A., Davey, R.P., Roberts, I.N., Burt, A., Koufopanou, V., Tsai, I.J., Bergman, C.M., Bensasson, D., O'Kelly, M.J., Van Oudenaarden, A., Barton, D.B., Bailes, E., Nguyen, A.N., Jones, M., Quail, M.A., Goodhead, I., Sims, S., Smith, F., Blomberg, A., Durbin, R., Louis, E.J., 2009. Population genomics of domestic and wild yeasts. Nature 458, 337–341. https://doi.org/10.1038/nature07743.

López-Uribe, M.M., Cane, J.H., Minckley, R.L., Danforth, B.N., 2016. Crop domestication facilitated rapid geographical expansion of a specialist pollinator, the squash bee Peponapis pruinosa. Proc. Biol. Scis. 283, 20160443. https://doi.org/10.1098/rspb.2016.0443.

Lorca, G., Uribe, S., Godoy, L., Ganga, M.A., Martinez Fernandez, C.A., Martinez Fernandez, C.A., 2018. Screening of native S. cerevisiae strains in the production of Pajarete wine: a tradition of Atacama Region, Chile. J. Wine. Res. 29, 130–142. https://doi.org/10.1080/09571264.2018.1465900.

Martínez, C., Cosgaya, P., Vásquez, C., Gac, S., Ganga, A., 2007. High degree of correlation between molecular polymorphism and geographic origin of wine yeast strains. J. Appl. Microbiol. 103, 2185–2195. https://doi.org/10.1111/j.13652672.2007.03493.x.

Martiniuk, J.T., Pacheco, B., Russel, G., Tong, S., Backstrom, I., Measday, V., 2016. Impact of commercial strain use on Saccharomyces cerevisiae population structure and dynamics in pinot noir vineyards and spontaneous fermentations of a Canadian winery. PLoS One 11, e0160259. https://doi.org/10.1371/journal.pone.0160259.

Naumov, G.I., Naumova, E.S., Sniegowski, P.D., 1998. Saccharomyces paradoxus and Saccharomyces cerevisiae are associated with exudates of north American oaks. Can. J. Microbiol. 44, 1045–1050.

Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U. S. A. 70, 3321–3323.

Ness, F., Lavallée, F., Dubourdieu, D., Aigle, M., Dulau, L., 1993. Identification of yeast strains using the polymerase chain reaction. J. Sci. Food Agric. 62, 89–94. https://doi.org/10.1002/jsfa.2740620113.

N'guessan, F.K., Djeni, N.T., Dadié, A.T., Djè, K.M., 2008. Influence of alcoholic fermentation temperature on sorghum beer quality. Food 2, 119–123.

N'guessan, F.K., N'Dri, D.Y., Camara, F., Djè, K.M., 2010. Saccharomyces cerevisiae and Candida tropicalis as starters cultures for the alcoholic fermentation of tchapalo, a traditional sorghum beer. World J. Microbiol. Biotechnol. 26, 693–699. https://doi.org/10.1007/s11274-009-0224-y.

N'guessan, F.K., Brou, K., Jacques, N., Casaregola, S., Djè, K.M., 2011. Identification of yeasts during alcoholic fermentation of tchapalo, a traditional sorghum beer from Côte d'Ivoire. Antonie Van Leeuwenhoek 99, 855–864. https://doi.org/10.1007/s10482-011-9560-7.

Nguyen, H.-V., Gaillardin, C., 1997. Two subgroups within the Saccharomyces bayanus species evidenced by PCR amplification and restriction polymorphism of the non-transcribed spacer 2 in the ribosomal DNA unit. Syst. Appl. Microbiol. 20, 286–294. https://doi.org/10.1016/S0723-2020(97)80075-6.

Peter, J., De Chiara, M., Friedrich, A., Yue, J.X., Pflieger, D., Bergström, A., Sigwalt, A., Barre, B., Freel, K., Llored, A., Cruaud, C., Labadie, K., Aury, J.M., Istace, B., Lebrigand, K., Barbry, P., Engelen, S., Lemainque, A., Wincker, P., Liti, G., Schacherer, J., 2018. Genome evolution across 1,011 Saccharomyces cerevisiae isolates. Nature 556, 339–344. https://doi.org/10.1038/s41586-018-0030-5.

Querol, A., Barrio, E., Huerta, T., Ramon, D., 1992. Molecular monitoring of wine fermentations conducted by active dry yeast strains. Appl. Environ. Microbiol. 58, 2948–2953.

R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Santiago-Urbina, J.A., Arias-García, J.A., Ruiz-Terán, F., 2015. Yeast species associated with spontaneous fermentation of taberna, a traditional palm wine from the southeast of Mexico. Ann. Microbiol. 65, 287–296. https://doi.org/10.1007/s13213-014-0861-8.

Schuller, D., Casal, M., 2007. The genetic structure of fermentative vineyard-associated Saccharomyces cerevisiae populations revealed by microsatellite analysis. Antonie Van Leeuwenhoek 91, 137–150. https://doi.org/10.1007/s10482-006-9104-8.

Sniegowski, P.D., Dombrowski, P.G., Fingerman, E., 2002. Saccharomyces cerevisiae and Saccharomyces paradoxus coexist in a natural woodland site in North America and display different levels of reproductive isolation from European conspecifics. FEMS Yeast Res. 1, 299–306. https://doi.org/10.1111/j.1567-1364.2002.tb00048.x.

Stringini, M., Comitini, F., Taccari, M., Ciani, M., 2009. Yeast diversity during tapping and fermentation of palm wine from Cameroon. Food Microbiol. 26, 415–420. https://doi.org/10.1016/j.fm.2009.02.006.

Tapsoba, F., Legras, J.-L., Savadogo, A., Dequin, S., Traore, A.S., 2015. Diversity of Saccharomyces cerevisiae strains isolated from Borassus akeassii palm wines from Burkina Faso in comparison to other African beverages. Int. J. Food Microbiol. 15, 30065–30069. https://doi.org/10.1016/j.ijfoodmicro.2015.07.010.

Techera, A.G., Jubany, S., Carrau, F.M., Gaggero, C., 2001. Differentiation of industrial wine yeast strains using microsatellite markers. Lett. Appl. Microbiol. 33, 71–75. https://doi.org/10.1046/j.1472-765X.2001.00946.x.

Tra Bi, C.Y., N'guessan, F.K., Kouakou, C.A., Noémie, J., Casaregola, S., Djé, M.K., 2016. Identification of yeasts isolated from raffia wine (Raphia hookeri) produced in Côte d'Ivoire and genotyping of Saccharomyces cerevisiae strains by PCR inter-delta. World J. Microbiol. Biotechnol. 32, 125–134. https://doi.org/10.1007/s11274-0162095-3.

Vezinhet, F., Blondin, B., Hallet, J.N., 1990. Chromosomal DNA patterns and mitochondrial DNA polymorphisms as tools for identification of enological strains of Saccharomyces cerevisiae. Appl. Microbiol. Biotechnol. 32, 568–571. https://doi.org/10.1007/BF0017 3729.

Viel, A., Legras, J.L., Nadai, C., Carlot, M., Lombardi, A., Crespan, M., Migliaro, D., Giacomini, A., Corich, V., 2017. The geographic distribution of Saccharomyces cerevisiae isolates within three Italian neighboring winemaking regions reveals strong differences in yeast abundance, genetic diversity and industrial strain dissemination. Front. Microbiol. 8, 1595. https://doi.org/10.3389/fmicb.2017.01595.

Yao, A., Egounlety, M., Kouamé, L.P., Thonart, P., 2009. Les bactéries lactiques dans les aliments ou boissons amylacés et fermentés de l'Afrique de l'Ouest: leur utilisation actuelle. Ann. Med. Vet. 153, 54–65.

Yu, J., Dossa, K., Wang, L., Zhang, Y., Wei, X., Liao, B., Zhang, X., 2017. PMDBase: a database for studying microsatellite DNA and marker development in plants. Nucleic Acids Res. D1046–D1053. https://doi.org/10.1093/nar/gkw906.