SELECTION OF INDIGENOUS *SACCHAROMYCES CEREVISIAE* STRAINS AS MIXED STARTER CULTURES FOR ALCOHOLIC BEVERAGE PRODUCTION IN CÔTE D'IVOIRE

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Abstract
The study of diversity and population structure of Saccharomyces cerevisiae isolated from Ivorian traditional alcoholic beverages, mainly sorghum beer and palm wines, revealed that different strains participate in the spontaneous fermentation. This study aimed to select a mixed starter culture of S. cerevisiae strains for beverage fermentation based on their positive interactions. Thus, 12 indigenous strains genetically typed by microsatellite analyses were tested in fermentation assays of pure cultures and mixed cultures of two and three strains. The fermentations were conducted with sorghum wort and oil palm sap and monitored by assessing yeast growth, total soluble solid (TSS) content, amount of CO_2 released, and volatile compounds. In pure cultures, the strains which demonstrated the best fermentative characteristics were YopI/2-2, BonVpA4, PG2-9 and AR1-3 in sorghum wort; AttIIIVp7, PG2-9, BonVpA7 and AR1-3 in oil palm sap. For co-inoculation of two strains in sorghum wort, the best starter cultures were MA6 (YopI/2-2 & AR1-1), MA2 (PG 2-9 & YopI/2-2) and MA7 (YopI/2-2 & AR1-3). In oil palm sap fermentation, MA3 (PG 2-9 & AR1-1) and MA4 (PG 2-9 & AR1-3) were the best starter cultures. A total of 38 and 43 volatile compounds were quantified when the three-strain starter cultures were used to ferment the sorghum wort and oil palm sap, respectively. The starters MB3 (BonVp A4 & PG 2-9 & AR1-1) and MB4 (BonVpA4 & AR1-1 & AR1-3) exhibited similar volatile profile whatever the starting material. So, they could be used as starter cultures for the production of alcoholic beverages.

Key words: Fermentation, Interaction, Oil palm sap, *Saccharomyces cerevisiae*, Sorghum wort, Starter culture

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INTRODUCTION

Traditional fermented beverages are an important part of the diet of African populations. Thus, various kinds of traditional alcoholic beverages are produced from many types of agricultural sources: rice, wheat, maize, barley, sorghum, millet, cassava, banana, palm sap, etc. These beverages are consumed during special ceremonies (marriage, dowry, birth) and many other happy or unhappy events. They have served to delineate social relations between family and group members, as well as among the elite and commoners, and to express a relationship between humans and deities (Dietlet, 2006). They exhibit several beneficial properties like attractive flavour, good digestibility, increased nutritional value, and a reduction of pathogenic microorganisms (Bourdichon et al., 2012). The production of these beverages goes through a stage of spontaneous fermentation involving a complex and variable natural flora, responsible for an inconstant final product from one production to another. To overcome this issue, the use of starter cultures was suggested as the appropriate approach (Tra Bi et al., 2021).

Several studies have been therefore conducted to determine the microbial species involved in the traditional fermented beverages in order to their use as starter cultures. In Côte d'Ivoire in particular, studies demonstrated that several species of lactic acid bacteria and yeasts are involved in the production of the main traditional alcoholic beverages, namely palm wine and sorghum beer (N'guessan et al. 2011; Tra Bi et al., 2016 ; Amoikon et al., 2018 ; Djéni et al., 2020). Among the yeast species, *Saccharomyces cerevisiae* was found as the

dominant species. The dominance of *S. cerevisiae* over other microbial competitors during alcoholic fermentations has been traditionally ascribed to its high fermentative power and aptitude to cope with the harsh environmental conditions, i.e.: high levels of ethanol and organic acids, low pH values, scarce oxygen availability and depletion of certain nutrients (Bauer and Pretorius 2000; Hansen et al. 2001).The functions attributed to this species in the production of alcoholic beverages would be related to the improvement of nutritional value, probiotic effects, inhibition of undesirable microorganisms and the simulation of lactic acid bacteria but also to the formation of alcohols and aromatic compounds (Jakobsen and Narvhus 1996; Jespersen 2003). But, the earlier studies aiming to develop starter cultures based on pure culture of *S. cerevisiae* strains resulted on beverages with poor flavour. Based on these results, cocultures consisting of different selected strains had been developed to produce products with stable and reproducible properties without a reduction in the concentration of key flavour substances. Most of this research has addressed the effect of simultaneous or sequential co-inoculation of non-*Saccharomyces* and *Saccharomyces* yeast strains or lactic acid bacteria and *Saccharomyces* yeast strains on traditional beverage flavour (Glover et al., 2009 ; N'guessan et al., 2010 ; Mukisa et al., 2017).

In the last decade, several authors highlighted the effect of co-fermentation using different *S. cerevisiae* strains on the aroma profiles of wine. Thus, Barrajon et al. (2011) demonstrated that the commercial *S. cerevisiae* strain was overtaken by a wild strain in a co-inoculated wine fermentation, and consequently modulated the flavour of the wine obtained. Blanco et al. (2011) found more than 40 different strains of *S. cerevisiae* among which 10 were found as the dominant strain or in codominance with other strains in spontaneous wine fermentations. Other studies described fermentation with mixtures of different strains of *S. cerevisiae* (Howell et al., 2006 ; Saberi et al., 2012 ; Capece et al., 2013 ; Perrone et al., 2013 ; Gustafsson et al., 2016), finding chemical differences in the wines produced and interaction between yeast strains involved in the co-inoculated fermentations. The competition degree of each strain is influenced by a number of abiotic factors (pH, temperature, ethanol, osmotic pressure, nitrogen, molecular sulphur dioxide, etc.) and biotic factors (microorganisms, killer factors, grape variety, etc.), which determine the capacity of one strain to out-compete another (Ciani et al., 2016).

The study of diversity and population structure of *S. cerevisiae* isolated from Ivorian traditional alcoholic beverages through nine regions revealed that different strains participate in the spontaneous fermentation, but some are dominant, and others are in the minority (Tra Bi et al., 2019). But no study reported on the effect of co-fermentation using different *S. cerevisiae* strains on the Ivorian traditional beverages. Here, we aimed to select *S. cerevisiae* strains as starter cultures for beverage fermentation based on their positive interactions.

MATERIALS AND METHODS

Yeast strains

Twelve indigenous *S. cerevisiae* strains belonging to the culture collection of the Food Technology Department (Nangui Abrogoua University, Abidjan, Côte d'Ivoire) and a reference strain (CLIB 154) obtained from CIRM-levure (France) were used in this study. Indigenous strains were isolated from sorghum beer (Yop I/ 2-2, TLM/ 1-8, Binger T/ A1-1, TPA 2-8), oil palm wine (Bon Vp-A4, Bon Vp-A7, Att PIII/ Vp7, PG 2-9) and raffia wine (AR 1-1, AR 1-3, GR 1-6, Adz R-C5). They were identified by molecular techniques (PCR-RFLP of the NTS2 region and sequencing of the D1/D2 domain of 26S rRNA gene) and genetically typed by microsatellite analyses (Tra Bi et al., 2019). The yeast strains were maintained routinely at −20°C in 20% of glycerol. For experiments, stock cultures were plated on YPD agar (1% yeast extract, 2% peptone, 2% glucose, 1.5% agar) and then incubated at 35°C for 24 h.

Fermentation assays

Pure cultures

Pure cultures were carried out in 250-mL Erlenmeyer flasks filled with 100 mL of fermentation medium, closed with dense cotton plugs and incubated at 35°C for 24 h with shaking at 150 rpm. The fermentation media used were liquid YPD, sorghum wort and oil palm sap. Sorghum wort and oil palm sap were obtained respectively from a local sorghum beer brewer at Adjame-Macaci and a palm wine tapper at Bingerville (district of Abidjan, Southern Côte d'Ivoire). They were sterilized by autoclaving at 100°C for 20 min. Each experiment was performed in triplicate. Yeasts were pre-cultured in each fermentation medium (liquid YPD, sorghum wort or oil palm sap) at 30°C for 48 h and used to inoculate the same fermentation medium at an initial concentration of 3.106 cells/mL. Fermentation progress were monitored throughout the fermentation process by assessing yeast growth (cell optical density, viable cell counts and dry weight) and total soluble solid concentration.

Mixed cultures

Five of the 12 indigenous *S. cerevisiae* strains were selected for their fermentation performance. A total of 14 mixed cultures were carried out as indicated in Table 1. Two combinations of mixed fermentations were examined in this study. The first combination examined the effect of a mixture of two *S. cerevisiae* strains (coded MA1 to MA10) at a ratio of 1:1 (cells/cells). The second combination examined the effect of a mixture of three *S. cerevisiae* strains (coded MB1, MB2, MB3 and MB4) at a ratio of 1:1:1 (cells/cells/cells). Fermentations were carried out in 250-mL Erlenmeyer flasks filled with 100 mL of sorghum wort or oil palm sap, autoclaved at 100°C for 20 min. Each sample was inoculated at 3.10^6 cells/mL with pre-cultures grown for 48 h at 30°C in the same fermentation medium. The flasks were covered with dense cotton plugs and incubated at 35°C for 24 h, 150 rpm. The experiments were replicated two times. Fermentation progress were monitored throughout the fermentation process by assessing yeast growth, total soluble solid concentration and the amount of CO_2 released. After fermentation, 50 mL beverage samples were placed in 50 mL glass vials with screw cap closures, refrigerated at 4°C to allow clarification and stored at −20°C until volatile compounds analysis.

Table 1 : Strain composition of yeast cultures used in fermentation trials

Fermentation trial	Combination of *Saccharomyces cerevisiae* strains
MA1	PG 2-9 & BonVp A4
MA2	PG 2-9 & YopI/2-2
MA3	PG 2-9 & AR1-1
MA4	PG 2-9 & AR1-3
MA5	YopI/2-2 & BonVp A4
MA6	YopI/2-2 & AR1-1
MA7	YopI/2-2 & AR1-3
MA8	BonVp A4 & AR1-1
MA9	BonVp A4 & AR1-3
MA10	AR1-1 & AR1-3
MB1	YopI/2-2 & PG 2-9 & AR1-1
MB2	YopI/2-2 & PG 2-9 & AR1-3
MB3	BonVp A4 & PG 2-9 & AR1-1
MB4	BonVpA4 & AR1-1 & AR1-3

Analytical determination

Typical determinations

The fermented samples (10 mL) were taken aseptically throughout fermentation. The yeast cell concentration was monitored by optical density at 600 nm (OD600); an OD600 of 1.0 corresponds to approximately 3.10^7 cells/mL. From these data, we fitted a growth population model with the growthcurver package in R (Sprouffske and Wagner, 2016) and determined maximum growth rate (μmax) for each fermentation.

Viable cell counts were evaluated by a traditional plate counting technique using Sabouraud-Chloramphenicol medium to estimate the total yeast populations. Plates were incubated for colony development at 30°C for 72 h.

For dry weight determination, the sample was first centrifuged (5 min at 5000 rpm) and the pelleted yeast re-suspended in sterile distilled water. The solution was centrifuged again under the same conditions to remove residual compounds. The supernatant was removed and the new pellet was diluted in 10 mL of sterile distilled water, dried on a thermo-balance using infrared rays.

The Total Soluble Solids (TSS) content was determined in each sample using a hand refractometer. The values were expressed in Brix as a total variation (ΔTSS).

CO_2 release during the fermentation process was followed by measurements of fermenter weight loss. The method described by Lai (2010) taking into account the volumes withdrawn during fermentation was used. The amount of CO_2 released allowed us to evaluate the maximum of released CO_2 (CO_2max expressed in g/L) which is the total amount of CO_2 released by the time fermentation is complete.

Headspace solid phase micro-extraction - gas chromatography/mass spectrometry (HS-SPME/GC-MS) analysis

The volatiles of fermented beverages were determined using a HS-SPME/GC–MS method. Around 3 g of each sample were placed in a sealed headspace vial of 10 mL. The HS-SPME extractions were carried out using 1 cm length fibers coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm) purchased from Supelco (Bellefonte, USA). All fibers were conditioned before analysis according to the manufacturer's instructions. The SPME procedure was performed automatically through the Agilent CTC PAL autosampler fitted to GC-MS instrument, consisting of the following sequential operations: sample conditioning under stirring for 10 min at 45°C, sample extraction for 20 min and injection by thermal desorption of the fiber. The isolation and identification of volatile compounds were carried out using a gas chromatography (Agilent model 7890B) equipped with an Agilent 5977A mass spectrometer detector. The volatiles were separated on a DB-5ms capillary column (30 m × 250 μm × 0.25 μm) with helium (≥99.999% purity) at a flow rate of 1.0 mL/min as the carrier gas. Splitless injection mode was adopted during volatile insertion at 250℃. The temperature program according was initially at 50℃ for 1 min and increased to 200℃ for 5 min at a rate of 3℃/min, then a ramp of 8℃/min to 150℃, and finally raised to 210℃ at 10℃/min hold for 5 min, which took 34.25 min in the entire procedure. The mass spectrometer was operated by electron impact (EI) method with an ionization energy of 70 eV and a source temperature of 230℃. Mass spectrometry uses full-scan mode with a mass range from m/z 30 to 400 m/z. The filament current and quadrupole temperature were 150 μA and 250℃, respectively. All measurements were performed three times. Analytical data was processed with Masshunter Profinder 8.0 software (Agilent Technologies, USA), while Mass Profiler Professional (MPP) v.15.0 software (Agilent Technologies, USA) was used as the chemometrics platform for alignment, and exploitation of the received MS data. MPP is the only platform that provides integrated identification/annotation of compounds and automated sample classification in combination with Masshunter.

Statistical analysis

All results are expressed as means ± standard deviations (n = 3). One-factor analysis of variance (ANOVA) and Tukey HSD (Honestly Significant Difference) tests were applied to the beverage composition data both independently for monocultures and for mixed cultures. The statistical software used was R 3.1.3 version and differences were considered significant for values of $P < 0.05$. Hierarchical clustering analysis (HCA) was performed with an R environment (http://www.r-project.org/) using the pheatmap package. A Principal Component Analysis (PCA) followed by a Hierarchical Cluster Analysis (HCA) was performed to classify the fermented beverages according to their volatile compounds. For HCA, Ward's criterion was set at 80% of homology.

RESULTS AND DISCUSSION

Fermentative characteristics of pure cultures

Table 2 shows the fermentative characteristics of the yeast strains isolated from traditional alcoholic beverages and the reference strain (CLIB 154). When the strains were cultured into the YPD medium, the maximum growth rate was 0.2 h^{-1} for all the strains, except for BonVpA4, BonVpA7 and PG2-9 which were isolated from palm wine. These strains demonstrated a higher maximum growth rate (0.3 h^{-1}). However, the strains that presented the highest dry matter were in order CLIB 154 (3 g/L), TPA/2-8 (2.7 g/L) and BonVpA4 (2.4 g/L). The strains PG2-9 and AdzRC5 had the lowest dry matter (1.9 g/L). Viable cell loads were statistically identical for all the strains and ranged from 7.1 to 7.7 log CFU/mL. The amount of total soluble solids (TSS) consumed was strain dependent. Indeed, the high values of 7.2 °Brix, 6.7 °Brix and 6.5 °Brix were observed respectively with the strains YopI/2-2, CLIB 154 and TPA/2-8. On the other hand, low variations of 1.2 °Brix, 1.4 °Brix and 3.8 °Brix were obtained with the strains AdzRC5, PG2-9 and BonVpA4, respectively.

When the strains were inoculated into the sorghum wort, seven strains showed maximum growth rates identical to the reference strain (0.1 h^{-1}) while five strains (BonVpA4, BonVpA7, PG2-9, AdzRC5 and AR1-3) gave higher values of 0.2 h^{-1} (Table 2). Strains that showed higher dry matter than the reference strain were YopI/2-2, PG2-9, TLM/1-8, BonVpA4, AR1-3, TPA/2-8 and AttpIIIVp7. With loads of 7.4, 7.3, 7.2 and 7.1 log (CFU/mL), BonVpA7, YopI/2-2, AR1-3 and PG2-9 showed the highest viable cell counts. All the strains, however, consumed a higher amount of TSS than the reference strain. The highest TSS values were obtained with strains GR1-6 (2.2 °Brix), YopI/2-2 (2.0 °Brix) and AttpIIIVp7 (2.0 °Brix).

In oil palm sap, all the strains showed a higher maximum growth rate than the reference strain, except for the strain AdzRC5 (Table 2). The highest value (0.5 h^{-1}) was observed with strains AR1-3, PG2-9 and BonVpA7. Dry matter values ranged from 3 g/L (AdzRC5) to 7.6 g/L (TLM/1-8). Only three strains (BonVpA4, AdzRC5 and TPA/2-8) showed lower values than strain CLIB 154. Viable cell loads were statistically identical for all the strains and ranged from 7.0 to 7.3 log (UCF/mL). However, the TSS consumed varied between strains. The strains AdzRC5, AttpIIIVp7 and PG2-9 showed, in order, the highest values of consumed TSS.

Differences in the fermentative characteristics observed here could be related to the strains genetic diversity and the different composition of the starting substrates. In fact, these strains were genetically typed by microsatellite analyses (Tra Bi et al., 2019) and selected based on their differences. The reasons for the difference between sugar consumption, as reported by Tra Bi et al. (2021), might be led to sugar transporters but also to the strains phosphorylation ability by gluco- and hexokinases, which corresponds to the first enzymatic step in the classical Embden-Meyerhof-Parnas glycolytic pathway. According to Tofalo et al. (2014), the yeast strain diversity might significantly affect the fermentation performance.

Table 2 : Growth and total soluble solid consumed by *Saccharomyces cerevisiae* strains on monoculture after 24 h of fermentation

Fermentation medium	Parameter	*Saccharomyces cerevisiae* strains												
		BingerT/A1-1	YopI/2-2	TLM/1-8	TPA/2-8	AttpIII Vp7	BonVpA4	BonVpA7	PG2-9	AdzRC5	AR1-1	AR1-3	GR1-6	CLIB 154
YPD	μ_{max} (h^{-1})	0.2± 0.0^{b}	0.2± 0.0^{b}	0.3± 0.0^{a}	0.2± 0.0^{b}	0.2± 0.0^{b}	0.3± 0.0^{a}	0.3± 0.0^{a}	0.3± 0.0^{a}	0.2± 0.0^{b}	0.2± 0.0^{b}	0.2± 0.0^{b}	0.2± 0.1^{b}	0.2± 0.0^{b}
	Dry weight (g/L)	2.0±0.1^{d}	2.0±0.0^{d}	2.0±0.0^{d}	2.7±0.0^{b}	2.2±0.1^{cd}	2.4±0.1^{c}	2.2±0.0^{cd}	1.9±0.0^{d}	1.9±0.1^{d}	2.1±0.0^{cd}	2.0±0.1^{d}	2.1±0.1^{cd}	3.0±0.0^{a}
	Yeast count (log CFU/mL)	7.2±7.1^{a}	7.6±6.4^{a}	7.5±5.0^{a}	7.4±6.2^{a}	7.5±6.4^{a}	7.7±7.6^{a}	7.4±7.3^{a}	7.5±6.5^{a}	7.1±7.0^{a}	7.3±6.6^{a}	7.1±7.0^{a}	7.4±7.2^{a}	7.2±6.7^{a}
	ΔTSS (°Brix)	5.0±0.2^{ab}	7.2±1.0^{a}	4.8±0.0^{ab}	6.5±1.2^{ab}	5.8±0.2^{ab}	3.8±1.7^{b}	6.2±0.7^{ab}	1.4±0.3^{c}	1.2±0.3^{c}	5.8±0.2^{ab}	5.5±0.0^{ab}	6.0±0.2^{ab}	6.7±0.0^{a}
Sorghum wort	μ_{max} (h^{-1})	0.1±0.0^{a}	0.1±0.0^{a}	0.1± 0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.2±0.0^{b}	0.2±0.0^{b}	0.2±0.0^{b}	0.2±0.2^{b}	0.1±0.0^{a}	0.2± 0.0^{b}	0.1±0.0^{a}	0.1±0.0^{a}
	Dry weight (g/L)	5.30±1.9^{e}	11.9±2.2^{a}	8.8±2.2^{c}	7.8±6.4^{c}	7.7±1.8^{c}	8.8±5.6^{c}	5.5±2.5^{e}	10.1±1.5^{b}	6.0±1.0^{d}	6.1±4.4^{d}	8.1±7.3^{c}	5.6±1.3^{e}	6.9±5.3^{d}
	Yeast count (log CFU/mL)	6.9±6.2^{b}	7.3±7.0^{ab}	6.7±6.3^{b}	6.5±5.6^{b}	6.4±6.0^{b}	6.9±6.7^{b}	7.4±7.0^{a}	7.1±6.6^{ab}	6.9±6.7^{b}	6.5±5.6^{b}	7.2±6.8^{ab}	7.0±6.0^{b}	7.0±6.4^{b}
	ΔTSS (°Brix)	1.9±0.0^{ab}	2.0±0.2^{ab}	1.7±0.0^{ab}	1.4±0.3^{ab}	2.0±0.2^{ab}	1.9±0.3^{ab}	1.9±0.0^{ab}	1.6±0.5^{ab}	1.8±0.2^{ab}	1.7± 0.3^{ab}	1.7±0.0^{ab}	2.2±0.0^{a}	1.2±0.0^{b}
Oil palm sap	μ_{max} (h^{-1})	0.2±0.0^{d}	0.3±0.0^{c}	0.3±0.0^{c}	0.3±0.0^{c}	0.4±0.0^{b}	0.2±0.0^{d}	0.5±0.02^{a}	0.5±0.0^{a}	0.1±0.0^{f}	0.3±0.0^{c}	0.5±0.0^{a}	0.2±0.0^{d}	0.1±0.0^{e}
	Dry weight (g/L)	6.0±0.0^{c}	5.8±0.0^{c}	7.6±0.0^{a}	4.8±0.1^{d}	5.9±0.0^{c}	3.1±0.0^{e}	5.4±0.1^{c}	5.3±0.1^{c}	3.0±0.1^{e}	5.7±0.0^{c}	6.8±0.1^{b}	5.3±0.1^{c}	5.3±0.5^{c}
	Yeast count (log CFU/mL)	7.1±7.0^{a}	7.1±6.8^{a}	7.0±6.8^{a}	7.1±6.9^{a}	7.2±7.0^{a}	7.3±7.1^{a}	7.2±7.0^{a}	7.2±7.0^{a}	7.1±6.9^{a}	7.3±7.1^{a}	7.2±7.0^{a}	7.3±7.0^{a}	7.1±6.9^{a}
	ΔTSS (°Brix)	1.7±0.3^{bc}	1.7±0.0^{bc}	0.8±0.2^{c}	2.0±0.2^{ab}	2.8±0.2^{ab}	2.2±0.3^{ab}	2.0±0.5^{ab}	2.6±0.0^{ab}	3.0±0.2^{a}	2.4±0.7^{ab}	1.9±0.0^{ab}	2.0±0.2^{ab}	2.3±0.2^{ab}

Data are means of three independant fermentations. Values not sharing the same superscript letter within a row are different according to the Tukey HSD test. YPD = a synthetic medium (1% yeast extract, 2% peptone, 2% glucose) ; ΔTSS = Total Soluble Solid consumed

Table 3 : Growth rate, total soluble solid consumed and CO_2 produced by mixed cultures (two strains) of *Saccharomyces cerevisiae* after 24 h of fermentation

Fermentation medium	Parameter	Fermentation trial									
		MA1	MA2	MA3	MA4	MA5	MA6	MA7	MA8	MA9	MA10
Sorghum wort	μ_{max} (h^{-1})	0.1±0.0^{b}	0.1±0.0^{b}	0.2±0.0^{a}	0.1±0.0^{b}	0.1±0.0^{b}	0.1±0.0^{b}	0.1±0.0^{b}	0.2±0.0^{a}	0.1±0.0^{b}	0.1±0.0^{b}
	ΔTSS (°Brix)	5.3±0.4^{ab}	7.1±0.4^{ab}	4.0±0.9^{ab}	3.2±1.2^{b}	7.2±1.1^{ab}	7.7±0.4^{a}	7.1±0.1^{ab}	5.5±1.8^{ab}	6.0±0.6^{ab}	5.1±1.0^{ab}
	CO_2max (g/L)	122.0±5.7^{a}	124.4±4.0^{a}	119.0±6.0^{a}	111.5±4.0^{a}	107.1±15.1^{a}	125.9±11.9^{a}	122±8.2^{a}	132.2±4.8^{a}	115.5±4.2^{a}	120.8±6.8^{a}
Oil palm sap	μ_{max} (h^{-1})	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	02±0.0^{a}
	ΔTSS (°Brix)	5.5±0.7^{a}	5.2±0.3^{a}	5.9±0.4^{a}	5.6±0.9^{a}	5.4±0.9^{a}	5.4±1.1^{a}	5.6±0.6^{a}	5.5±0.7^{a}	5.4±0.9^{a}	5.5±0.7^{a}
	CO_2max (g/L)	129.4±9.3^{a}	135.7±10.0^{a}	135.6±9.8^{a}	137.0±10.1^{a}	132.1±9.7^{a}	132.3±9.7^{a}	129.5±9.4^{a}	129.4±9.3^{a}	130.3±9.5^{a}	132.1±9.5^{a}

Data are means of two independant fermentations. Values not sharing the same superscript letter within a row are different according to the Tukey HSD test. MA1, MA2, ... MA10 are a mixture of two Saccharomyces cerevisiae strains; ΔTSS = Total Soluble Solid consumed

The pheatmap analysis carried out on the basis of the four parameters studied showed that in the YPD medium, the strains that demonstrated the best fermentative characteristics were BonVpA4, BonVpA7 and TLM/2-8 (Figure 1A). In the sorghum wort, five clusters were defined. The reference strain alone forms one cluster. The strains YopI/2-2, BonVpA4, PG2-9 and AR1-3 showed the best fermentative characteristics in this medium (Figure 1B). Five clusters were also defined when the strains were inoculated into oil palm sap. The strains that showed the best characteristics are grouped in the cluster composed of AttIIIVp7, PG2-9, BonVpA7 and AR1-3 (Figure 1C).

Fermentative characteristics of mixed cultures of two strains

Ten mixed starters consisting of two *S. cerevisiae* strains were formulated from the five strains selected from the pure cultures (YopI/2-2, PG2-9, BonVpA4, AR1-3, AR1-1). As shown in Table 3, the highest maximum growth rate was 0.2 h^{-1}, in contrast to monocultures where values of 0.5 h^{-1} were observed. This result reflects that co-culture does not induce an increase of the maximum growth rate. On the contrary, it reflects that one strain was outnumbered by another in the mixture. This is known as ‘’intra-species dominance’’, a phenomenon often observed in alcoholic fermentation. The reason for this was not investigated but according to the literature, the dominance could be attributed to competition (for nutrients, space), differences in fitness (resistance to ethanol or other metabolites) or cell-to-cell contact and aggregation which are driven by the expression of genes that are associated with the cell surface (Pérez-Torrado et al., 2017). Ciani et al. (2016) reported on a number of abiotic factors (pH, temperature, ethanol, osmotic pressure, nitrogen, molecular sulphur dioxide, etc.) and biotic factors (microorganisms, killer factors, grape variety, etc.), which determine the capacity of one strain to out-compete another on wine fermentation.

Interaction among different yeast species or yeast strains can have either stimulatory or inhibitory effects on their metabolic activity. In this study, the interaction could not result from CO_2 production as all the mixed starters showed statistically identical CO_2 contents for each starting material. The values were located between 107.1 g/L and 132.2 g/L and between 129.4 g/L and 137.0 g/L, respectively for sorghum wort and oil palm sap (Table 3).

The most important factor in the selection of a yeast strain is the consumption of sugar (Feghali et al., 2019). With oil palm sap, the mixed cultures showed similar trends in sugar consumption with TSS variation of 5.2-5.9 °Brix (Table 3). But with sorghum wort, the starters MA2, MA5, MA6 and MA7 demonstrated the highest TSS consumption of 7.1-7.7 °Brix. All these mixed starters shared the strain YopI/2-2 isolated from sorghum beer which in pure culture consumed only 2 °Brix of TSS.

Table 4 : Growth rate, total soluble solid consumed and CO_2 produced by mixed cultures (three strains) of *Saccharomyces cerevisiae* after 24 h of fermentation

Fermentation medium	Parameter	Fermentation trial			
		MB1	MB2	MB3	MB4
Sorghum wort	μ_{max} (h^{-1})	0.2± 0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}
	ΔTSS (°Brix)	5.9±0.0^{a}	5.8±0.0^{a}	5.6±0.0^{a}	5.7±0.0^{a}
	CO_2max (g/L)	127.4±0.6^{a}	126.3±0.3^{a}	125.2±1.5^{a}	126.7±0.3^{a}
Oil palm sap	μ_{max} (h^{-1})	0.2±0.0^{b}	0.3±0.1^{a}	0.3±0.0^{a}	0.2±0.0^{b}
	ΔTSS (°Brix)	5.6±0.9^{b}	5.8±0.6^{ab}	7.9±0.4^{a}	7.8±0.0^{ab}
	CO_2max (g/L)	107.0±2.6^{b}	108.0±3.6^{b}	120.0±0.6^{a}	121.3±0.8^{a}

Data are means of two independant fermentations. Values not sharing the same superscript letter within a row are different according to the Tukey HSD test. MB1, MB2, MB3 and MA4 are a mixture of three *Saccharomyces cerevisiae* strains ; ΔTSS = Total Soluble Solid consumed

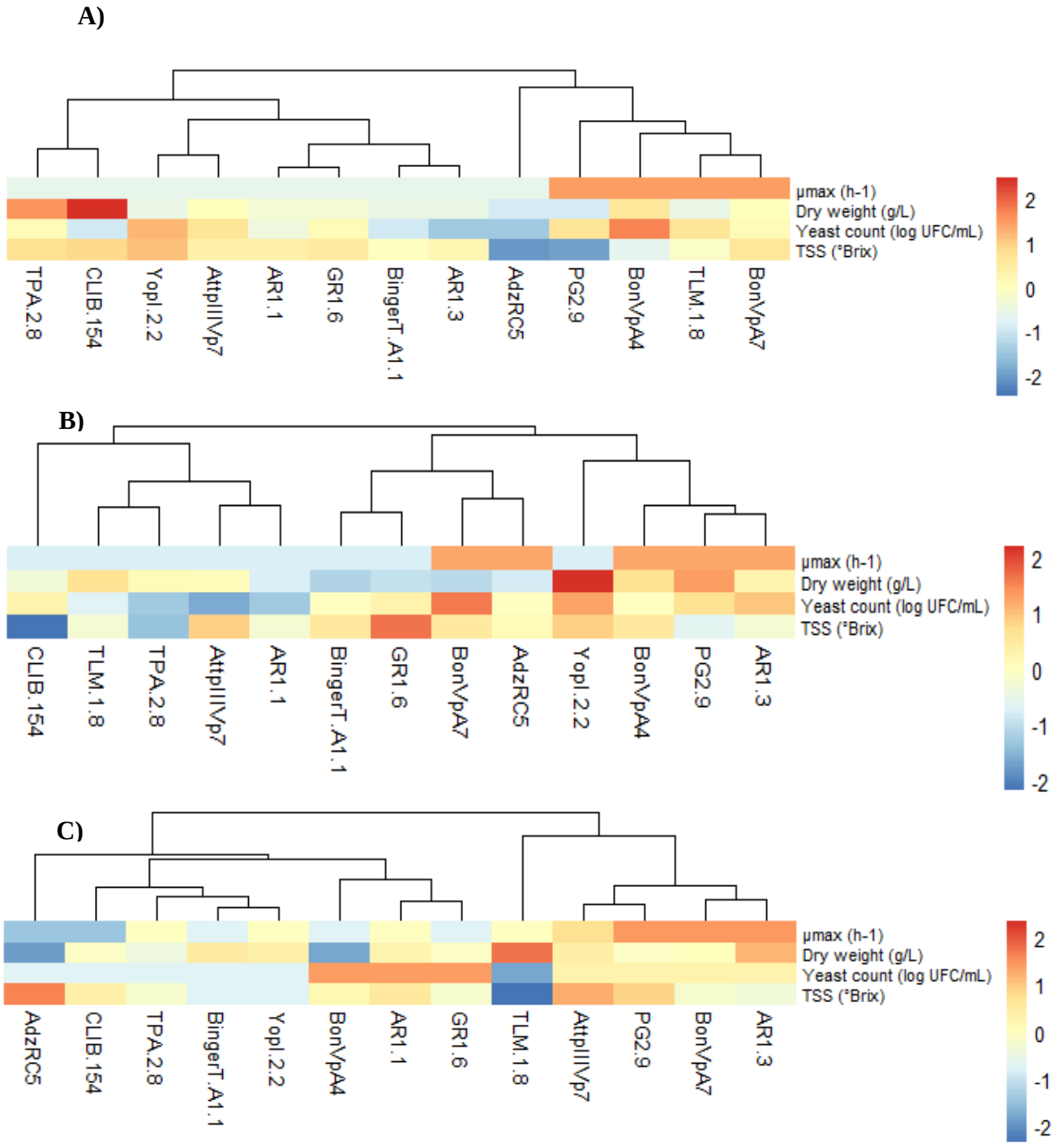

Figure 1 : Cluster analysis of fermentative characteristics of *Saccharomyces cerevisiae* strains pure cultures using the pheatmap package in the R software
A : strains inoculated into YPD medium ; B : strains inoculated into sorghum wort ;
C : strains inoculated into oil palm sap

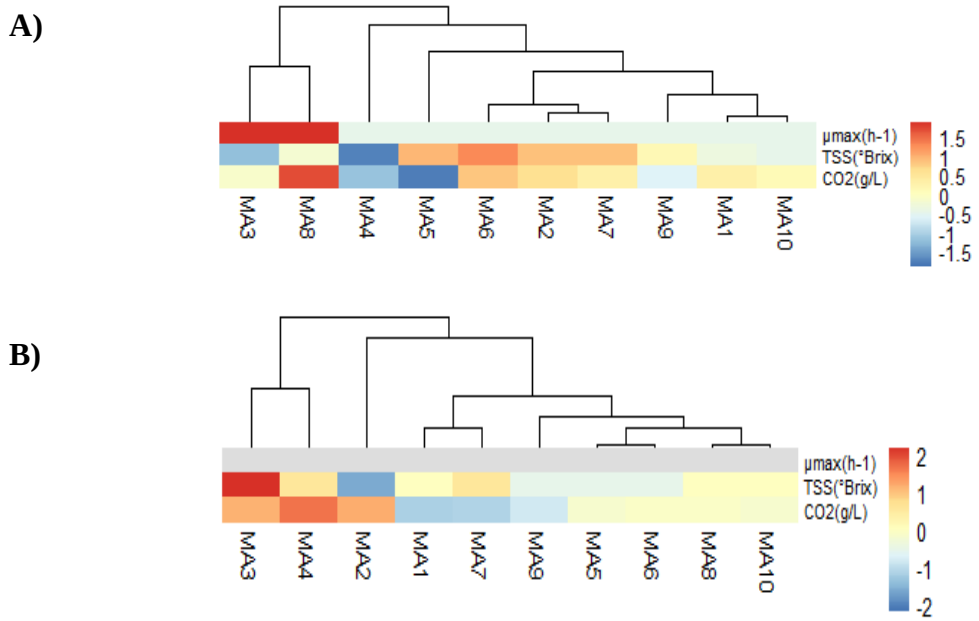

Figure 2 : Cluster analysis of fermentative characteristics of *Saccharomyces cerevisiae* mixed cultures of two strains using the pheatmap package in the R software
A : strains inoculated into sorghum wort ; B : strains inoculated into oil palm sap

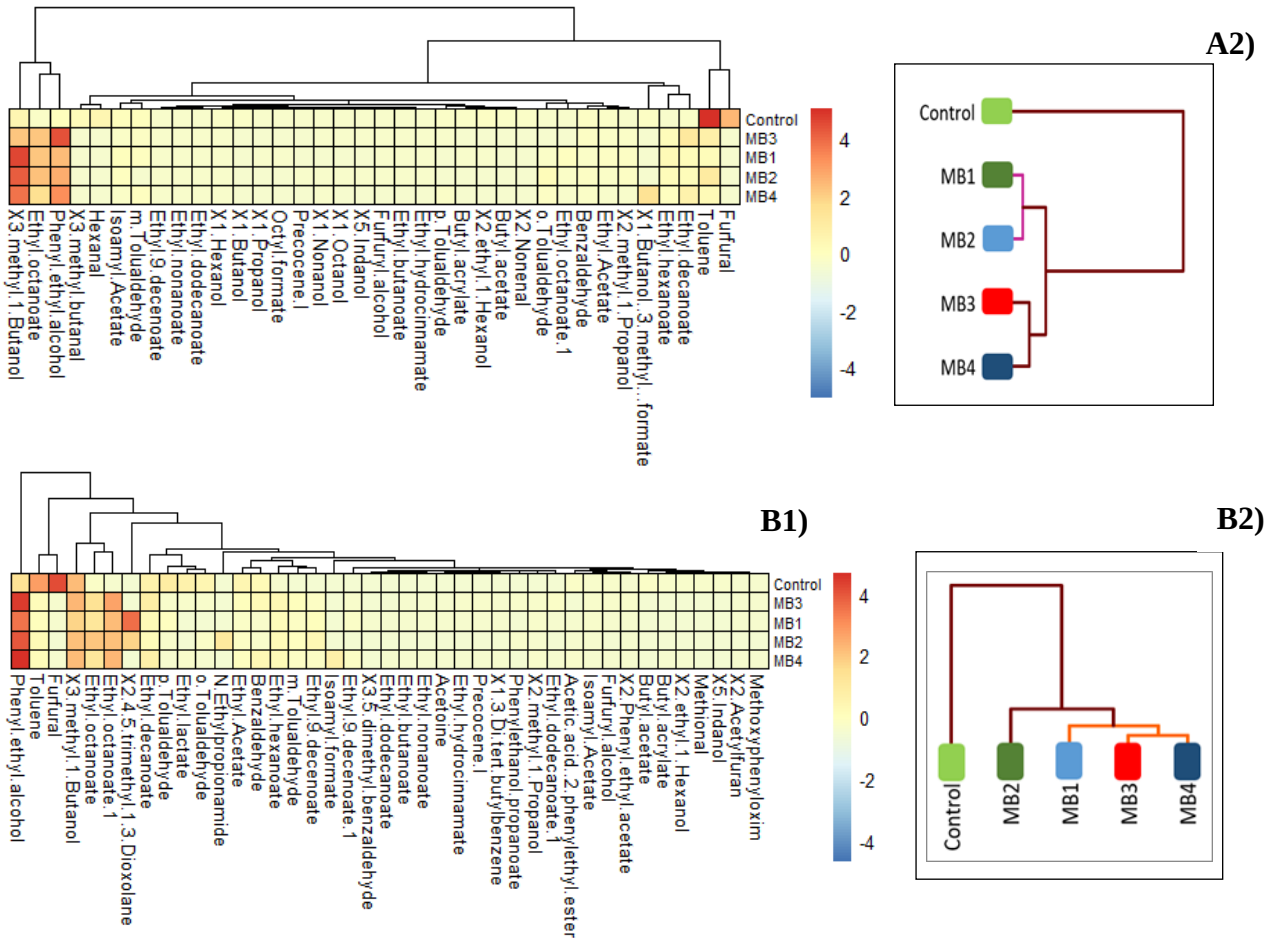

Figure 3: Pheatmap analysis and hierarchical classification of *Saccharomyces cerevisiae* mixed cultures of three strains based on volatile compounds
A : strains inoculated into sorghum wort ; B : strains inoculated into oil palm sap

These results indicate positive metabolic interaction between component strains of starter cultures, mainly between the strain YopI/2-2 and the others. The positive influence of yeast strains on certain basic fermentation parameters such as sugars consumption, alcohol content, total and volatile acidity have been extensively reported (Rojas et al., 2012; Blanco et al., 2014).

The pheatmap analysis showed that the best starter cultures in sorghum wort fermentation are MA6, MA2 and MA7 (Figure 2A). In oil palm sap fermentation, MA3 and MA4 were shown as the best starter cultures (Figure 2B).

Fermentative characteristics of mixed cultures of three strains

As the best fermentative mixed starter composed of two strains varied according to the fermentation medium, we tested mixed cultures of three strains. With a maximum growth rate of 0.2 h^{-1} on sorghum wort and 0.2-0.3 h^{-1} on oil palm sap L (Table 4), the results confirmed that co-culture does not induce an increase of the maximum growth rate. Besides, a potential metabolic interaction among strains included in mixed starter cultures of two strains seems to be confirmed by the results of mixed starter cultures of three strains. In fact, relative to the monoculture, mixed starter cultures of three strains showed higher TSS consumption of 5.6-5.9 °Brix and 5.5-7.9 °Brix for sorghum wort and oil palm sap, respectively (Table 4). This result add further weight to the statement that yeasts can modify the products of fermentation when grown in mixed culture.

The literature reported that yeasts modify their metabolism during growth in mixed fermentation, where interaction among strains composing mixed starter cultures can determine sharing of some secondary metabolites (Howell et al., 2006; King et al., 2008). In this study, the analysis of volatile compounds, considered as a partial or volatile-fraction 'metabolome', showed that 38 and 43 compounds were found in fermented sorghum wort and oil palm sap, respectively (Figure 3). They belong to four chemical classes (esters, alcohols, aldehydes, and other compounds). This is in agreement with Amoikon et al. (2020) who reported 50 volatile organic compounds produced by yeast strains isolated from palm wine among which esters were the most abundant (28 compounds). Furthermore, Attchelouwa et al. (2020) identified 63 compounds in fresh sorghum beer produced from indigenous starter, indicating that the mixed starters tested in this study synthesized most of this traditional beer flavour compounds. On the other hand, the number of volatile compounds found in each fermented product was ranged between 23 and 35 with product obtained from the starter MB4 displaying the highest number. These compounds, according to Bordet et al. (2020), can be produced by yeasts metabolizing sugars and amino acids. Phenyl ethyl alcohol, ethyl octanoate and 3-methyl-1-butanol were found as the majors volatile compounds. Several authors reported also ethyl octanoate (fruity, apricot and banana notes) among esters that have a significant impact on the aroma profile of an alcoholic beverage (Welke et al., 2012; Garofalo et al., 2018). For alcohol compounds, it has been reported that ethanol and phenyl ethyl alcohol impart the aroma of alcoholic beverages by exhibiting a bitter taste, a rose like essence respectively (Ho et al., 2013). The HCA based on volatile compounds showed that the starters MB3 and MB4 exhibited similar volatile profile whatever the starting material on contrary to the others. These results might be due to the strains composition. In fact, the starters MB3 and MB4 were composed of three strains isolated from palm wines (raffia and oil palm wines) in contrast to MB1 and MB2 which were constituted from strains isolated from sorghum beer (strain YopI/2-2) and palm wines (strains PG2-9, AR1-1 and AR1-3).

CONCLUSION

Strains used as starter cultures in alcoholic fermentations go through several levels of selection. In this study, we tested *S. cerevisiae* strains isolated from various substrates first in monoculture and then in coculture or mixed culture of two and three strains in the

fermentation of sorghum wort and oil palm sap. The results demonstrated that the best fermentative mixed starter composed of two strains varied according to the fermentation medium. But with mixed culture of three strains, starters MB3 and MB4 showed the best fermentative characteristics as well as more interesting odour profiles whatever the starting material. Thus, they could be used as starter cultures for the fermentation of sorghum wort and oil palm sap as well as many other substrates for the production of alcoholic beverages.

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