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Characterization of *Saccharomyces cerevisiae* Strains from Palm Wine for Baking Leaven Production in Republic of Congo

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Abstract This study was carried out to select the autochthon yeast strains from the palm wine. The work aims to wildly isolate, characterize and identify *Saccharomyces cerevisiae* in order to locally and safely produce a bio-leaven and to assess their ability to ferment wheat flour dough used for the bread making and other products. Palm wine samples have been bought in the village around Brazzaville. Samples have been collected in sterile glass bottles then stored under refrigeration condition at 4°C until they were processed. Yeasts were enumerated and isolated on Dichloran Rose Bengal Chloramphenicol agar medium at 37°C. Molecular identification was carried out by a PCR-based method using species-specific primer pairs to *Saccharomyces* genus without sequencing stage. The results showed that palm wine contains about 3.310^6 $CFUmL^{-1}$. Fifty-one isolates were obtained with a percentage of 13% corresponding to *Saccharomyces cerevisiae*. This identity was confirmed by the molecular characterization. Five yeast strains showed high ability to ferment wheat flour dough analogous of the control. The better growth of isolates was between 28 and 37°C. However, the near neutral pH gives the higher growth. High concentration of NaCl and glucose in medium has a strong negative impact on the growth of isolates.

Keywords: *palm wine, Saccharomyces cerevisiae, bio-leaven, wheat, dough, PCR-based, NaCl*

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1. Introduction

The food practices in the Congolese cities seems to be shifted to others eating habits. Indeed, in many Congolese families the manufactured products containing wheat flour including bread, fritters and cakes are gradually replaced by the time the local food. These food products resulting from fermented wheat flour dough used commonly as additives of breakfast, lunch dinner are currently substituted with traditional products including manioc roots, sweet potato, yams and wheat. Doughs are obtained by incorporating into the wheat flour of bakers' yeasts. The biological leavens are mainly *Saccharomyces cerevisiae* strains. In Republic of Congo leavens are imported. Indeed, there are not any industries producing this significant input into the good manufacture practices of bakery. As consequent the price of bread is still increasing in two couple of cities including Pointe-Noire (10 fold) and Brazzaville (15 fold) basing for 30 years ago. This could be keep on increasing as long as Republic of Congo will not proactively commit to safely produce inputs used in different preparations of doughs.

Microorganisms like *Saccharomyces cerevisiae* strains are wildly used as leaven in sheets and fermented food. Several authors have been isolated *Saccharomyces cerevisiae* from fermented corn paste [1,2,3], from fermented cassava roots and sheets [4,5], and from palm wine [6,7,8]. The local beverages such as the palm wine contain yeasts of *Saccharomyces* genus [7,9] *Saccharomyces cerevisiae* is able to degrade glucose by producing ethanol and carbon dioxide in anaerobic condition. However, the production of carbon dioxide is the one of criteria making possible to raise the flour dough [10]. Basing on some fermented food and beverages found in Republic Congo [9], this work aims to wildly isolate, characterize and identify *Saccharomyces cerevisiae* in order to locally and safely produce a bio-leaven and to assess their ability to ferment wheat flour dough used for the bread making and other products. This study was carried out to select the autochthon yeast strains from the palm wine with the abilities to be used in the fermentation of the wheat flour dough.

2. Materials and Methods

2.1. Materials Origin

In this study, three palm wine samples have been bought from three main markets in a couple of districts of Brazzaville as indicated in Table 1. Samples have been collected in sterile glass bottles then stored at 4°C until they were processed.

Table 1. Origin and nature of palm wine

Sample code	Nature	Origin of palm win
P1	palm wine	NGanga-Lingolo
P2	palm wine	Madibou
P3	palm wine	Djiri

2.2. Yeast Numeration

Yeasts were enumerated on Dichloran Rose Bengal Chloramphenicol agar medium (DRBC). One mL of palm wine sample was diluted in 9mL physiologic water contained into test tube then mixed with vortex (VELP Classic Advanced Vortex Mixer). This corresponded to a 10^{-1} dilution. Decimal dilutions were prepared from this suspension by inoculating 1 mL of 10^{-1} suspension in test tubes containing 9mL physiologic water. Then Petri dishes containing culture DRBC agar medium were sowed with 0.1 mL of dilutions 10^{-1} to 10^{-6}. The sowings were carried out in surface and aerobic incubations at 30°C for 4 to 6 days. Three Petri dishes were used by dilution. DBRC agar medium contained per liter distilled water 5g of soybean peptone, 10g of dextrose, 1.0g of Monopotassium phosphate, 0.5 magnesium sulfate, 0.025 g of rose-bengal, 0.002g of dichloran, 0.1 g of chloramphenicol, and 15 g/L of agar-agar. After incubation the colonies were counted manually and results were expressed in colony forming unit per milliliter (CFU/mL).

2.3. Isolation and Characterization of Palm Wine Yeasts

At the incubation end the petri dishes showing of separated colonies were retained for isolation and purification of strains. Isolated colonies were purified by repeated streaking onto DRBC agar until to obtain identical colonies. Cultural characteristics were assessed by examining the aspect of the liquid culture in particular basing on turbid, including the presence of a veil on the surface of YPG broth, and presence of a deposit at the test tube bottom. Cells morphologic characterization was examined under light microscope (Paralux optical microscope, L1100 S2 Trino-1600x, France) at 400x magnification into see cells shape, budding type, and presence of pseudo mycelium or mycelium. The composition for YPG broth is as follows: 1 g/L of yeast extract, 5 g/L of peptone, and 2.5 g/L of glucose (pH 6.5).

2.4. Production Test of Gas

The production test of gas was assessed according to Merabti [11] and Dieng [12] to know if the isolates obtained are able to produce gas. Inoculation was done in a test tube containing 9 mL of liquid YPG medium and an inverted Durham tube. Test tube was sown with a yeast colony of 24H old. The culture was incubated at 37°C during 2 to 7 days. At the end of the incubation period, the presence of a vacuum in the Durham tube put in evidence the gas production. The experiment was carried out in triple.

2.5. Study of Wheat Flour Dough Leavening Capacity of Yeast Isolates

2.5.1. Leaven Preparation

1 mL of an overnight culture was inoculated in 250 mL of previously sterilized PEG medium, and then the bottle was incubated at 28°C during 48 hours [13]. After 48 hours cultures were centrifuged at 5000 rpm during 10 min (Eppendorf centrifugeuse 5805, Allemagne). The pellet was recovered and washed with physiological water. The mix was centrifuged at 5000 rpm during 20min. This operation was done in triplicate. Then pellet was recovered to be used as leaven raw.

2.5.2. Preparation of the Dough

Composition of ingredients used in the preparation of wheat flour dough is shown in Table 2. In 1L flask, 20 mL of sterile water, 25 g of wheat flour, 0.45 g of salt, and 0.375 g of leaven raw previously prepared were added (Table 2). Then the mixture is homogenized by kneading using a sterile spatula. Flask has been closed and incubated at 28°C for 5 hours. Dough pH was determined at the beginning and at the end of the fermentation using a ROHS pH meter (China).Positive control has been performed by inoculating the dough with the *Saccharomyces cerevisiae* baker's yeast (boughtto market) while in negative control no inoculum was added. Experience was carried out in triplicate.

Table 2. Ingredients used in fermented dough preparation of wheat flour

Palm wine		Ingredients of fermented dough	
Sample code	Origine	Composition	Quantity %
P1	NGanga-Ligolo	Wheat flour	100
P2	Madibou	Salt (NaCl)	1.8
P3	Djiri	Leaven	1.5
-	-	Water	67

2.6. Identification of Yeast Isolates

Biochemical characterization has been performed by setting 20 yeast identification tests using Analysis Profile Index test strips (API 20 C AUX, BioMerieux, France) according to the manufacturer's instructions. Briefly, using a micropipette, a fraction of 24 H-old colony was collected by suction and transferred into one API C Medium ampoule. The mix was homogenized with the micropipette. The microtubes containing dehydrated substrates were inoculated with the suspension obtained by placing the tip of the micropipette on the side of the microtube avoiding bubbles formation. Then incubation box containing the inoculated API 20 C AUX strip was closed and incubated

at 37°C for 24 to 72 hours. During incubation, the yeast metabolism results in a growth revealed by the turbid of the medium in microtubes. After incubation period, the positive and negative reactions in each strip were analyzed using the Reading table provided. On the results sheets, the tests were separated into groups of 3 and a value of 1, 2 or 4was assigned for each positive reaction, in accordance with the manufacturer's instructions. Adding together the values for a positive reaction for each test within each group gives a 7-digit profile number for the 20 tests in the API 20 C AUX strip. The yeasts were identified by referring the profile number in the Analytical Profile Index.

2.7. Molecular Identification

Molecular identification was carried out by a PCR-based method using species-specific primer pairs to *Saccharomyces* genus without sequencing stage [14]. Names of each orthologous gene targeted, species specific primer sequences and their sizes are shown in Table 3. The total genomic DNA of yeast strains was extracted by using Nucleo Spin® Microbial DNA extraction kit (Macherey-NAGEL) according to the manufacturer's guidelines. Then DNA obtained was controlled by 1% agarose gel electrophoresis in 0.5x TAE buffer and purified using E.Z.N.A. Gel extraction kit (OMEGA biotek) according to the manufacturer's instructions. The specific genes at each species *Saccharomyces* were amplified separately by polymerase chain reaction (PCR) in a 50 µL reaction mixture (total volume) containing 1.25 U of Taq polymerase (Eurogentec, Seraing, Belgium), 200 µM of mix dNTP, 2 µM of specific primer, 2ng/µL of DNA, 10 µL of 10X Taq polymerase buffer and 31.75 of sterile distilled water. The PCR was carried out using a thermocycler (Bio-RAD T100^{TM}) with an initial denaturation for 5min at 95°C; 25 cycles of 30 s at 95°C, 30 min at 55°C and 40 s at 72°C, and a final extension for 10 min at 72°C. The PCR products were run on 1% (wt/vol) agarose- 0.5X TAE (Tris, Acetate, EDTA) buffer gel stained with Bromide of ethidium 100 V for 30 min and the migration patterns of amplicons were visualized under ultraviolet light with the lamp (BioRad, Richmond, California, USA).

2.8. Effect of Abiotic Factors on Strains Growth

Yeast strains that displayed high ability to raise dough compared with positive control were selected for to study influence of abiotic factors on isolates growth.

2.8.1. Temperature

A 0.1 mL volume of 24 H-old fresh culture of purified strain was taken and inoculated in test tube containing 9 mL of liquid YPG medium. Test tube was incubated at room temperature, 37°C, 44°C, and 50°C during 24 hours. Then strain growth was estimated by measuring optical density of culture at 600 nm using a spectrophotometer (model 722, visible spectrophotometer). Experience was carried out in triplicate.

2.8.2. pH

To evaluate pH effect on the strains growth, liquid YPG medium was prepared and pH was adjusted with NaOH (1M) or HCl (1M) at 3, 5, 7 and 9. Then a volume of 0.1 mL of 24 H-old fresh culture was taken and inoculated separately in test tubes containing 9 mL YPG medium previously prepared. An overnight culture was incubated at 37°C. The growth was evaluated by measuring optical density of culture at 600 nm using a spectrophotometer (model 722, visible spectrophotometer) and this was carried out in triplicate.

2.8.3. Sodium chloride (NaCl) and Glucose

Purified isolates were cultivated in liquid PEG medium to concentrations different of NaCl (0g/L, 1.8g/L, 3.6g/L and 5.4g/L). The growth was controlled by measuring the optical density after 24 hours of incubation at 600 nm using a spectrophotometer. Three test tubes were inoculated per isolate. Liquid PEG medium was prepared in varying content in glucose (0%, 6%, 12% and 18%). Then a volume of 0.1 mL of purified strain pre-cultivation was taken and inoculated in test tubes containing culture medium. Cultures were incubated at 37°C during 24 hours. The growth is evaluated as described previously.

Table 3. Species specific primer sequences and sizes [14]

Species	Primer sequence (5' → 3')	Primer name	Amplicons size (pb)
Saccharomyces cerevisiea	GCG-CTT-TAC-ATT-CAG-ATC-CCG-AG AA-GTT-GGT-TGT-CAG-CAA-GAT-TG	ScerF2 ScerF2	150
Saccharomyces arboricolus	GGC-ACG-CCC-TTA-CAG-CAG-CAA TCG-TCG-TAC-AGA-TGC-TGG-TAG-GGC	Sarb_F1 Sarb_R2	349
Saccharomyces bayanus	GCT-GAC-TGC-TGC-TGC-TGC-CCC-CG TGT-TAT-GAG-TAC-TTG-GTT-TGT-CG	Sbay_F1 Sbay_R1	275
Saccharomyces kudriavzevii	ATC-TAT-AAC-AAA-CCG-CCA-AGG-GAG CGT-AAC-CTA-CCT-ATA-TGA-GGG-CCT	Skud_F2 Skud_R1	660
Saccharomyces Mikatae	ACA-AGC-AAT-TGA-TTT-GAG- GAA-AAG CCA-GTC- TTC-TTT- GTC-AAC- GTT-G	Smik_F1 Smik_R1	508
Saccharomyces paradoxus	CTT-TCT-ACC-CCT-TCT-CCA-TGT-TGG CAA-TTT-CAG-GGC-GTT-GTC-CAA-CAG	Spar_F7 Spar_R7	739

3. Results

3.1. Numeration, Isolation and Characterization of Yeasts Isolates

Figure 1 shows that three samples have a density about 10^6. Indeed, yeasts concentration in the samples P1, P2 and P3 are respectively 1.6 10^6, 0.3 10^6 and 3.3 10^6 $CFUmL^{-1}$. From enumeration petri dishes, 51 yeast isolates were purified. Then microscopic analysis of these 51 purified isolates was carried out, yet only 13% of isolates resembling *Saccharomyces* genus were retained. Figure 2a and Figure 2b show the colonies of S7P1 isolate and their cellular shape using microscope. The others isolates resembling at Candida genus were systematically discarded. Table 4 shows the results morphological observation of 13% of selected isolates. All isolates have a multilateral budding, with cells oval and spherical or round except S1P3 isolate that has lengthened cells, no veils on surface but fine deposit formation at bottom of culture tube and absence of mycelium except S1P3 isolate that forms pseudo-mycelium. Subcultures were carried out each month to maintain the isolates. These isolates were preserved in double on malt extract slant agar medium in test tube at ambient temperature.

3.2. Assessment of CO_2 Production

Table 5 show the results of gas production in liquid YPG medium by purified isolates retained. Only isolate S1P3 produces a quantity of gas comparable with the positive control after 5 H incubation at 37°C. On the other hand, for S7P1, S14P1, S18P1 and S15 P2 isolates the production is very low represented by one bubble in Durham bell. However, S14P2 and S20P2 isolates did not produce gas. Figure 3 illustrated gas production of S7P1 isolate.

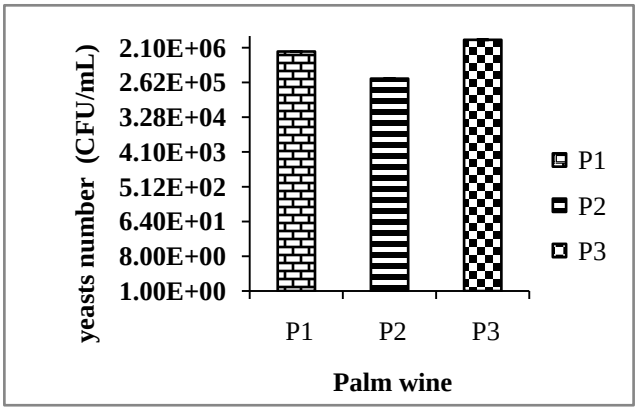

Figure 1. Yeasts numeration in three palm wine samples P1, P2 and P3

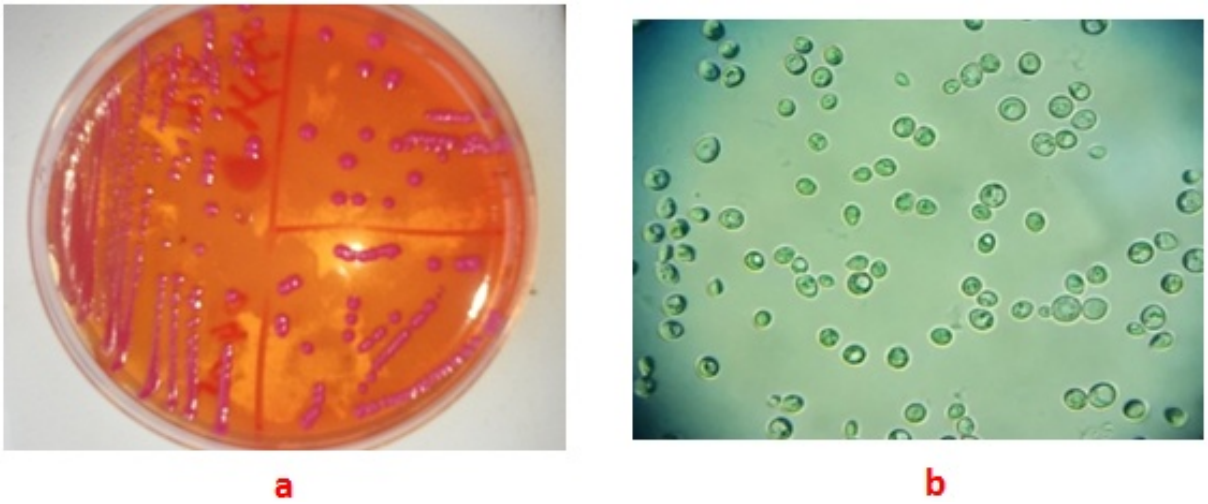

Figure 2. Purified colonies (a) of S7P1 isolate on RBDC agar and cells shape (b) at microscope at GX40

Table 4. Morphologic and cultural characterization of purified yeasts isolate from palm wine

Isolate	Budding type	Cell shape	Veil on surface	Deposit formation	Mycelium
S7P1	Multilateral	Oval and spherical	No	Yes	No present
S14P1	Multilateral	Ooval and spherical	No	Yes	No present
S18P1	Multilateral	Oval and round	No	Yes	No present
S14P2	Multilateral	Oval and round	No	Yes	No present
S15P2	Multilateral	Oval and spherical	No	Yes	No present
S20P2	Multilateral	Oval and round	No	Yes	No present
S1P3	Multilateral	Spherical and lengthened	Yes	Yes	Pseudomycelium

Table 5. Test of gas production by yeast isolates

Sample	P1			P2			P3	*Saccharomyces cerevisiae*
Isolate	S7P1	S14P1	S18P1	S14P2	S15P2	S20P2	S1P3	T (+)
Gas production	±	±	±	-	±	-	++	++

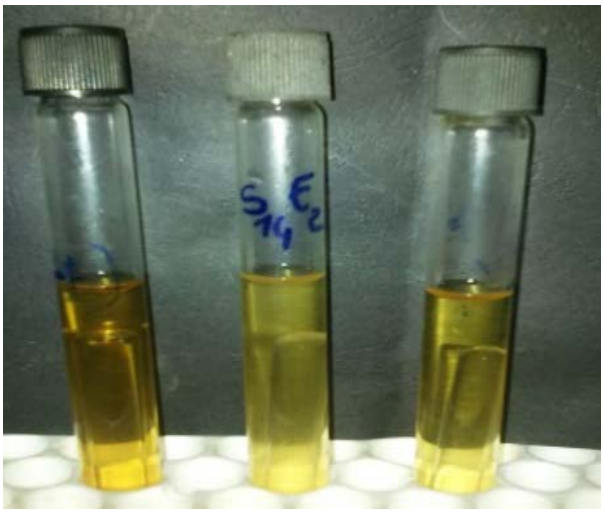

Figure 3. Test of gas production in liquid PEG medium, of left on right S7P1, S14P2, and S20P2 isolates

3.3. Yeasts Capacity to Ferment the Wheat Flour Dough

Each of seven isolates was tested for their capacity to ferment the wheat flour dough. Table 6 shows that five isolates S7P1, S18P1, S14P1, S14P2, and S20P2 fermented the wheat flour dough after 5 H incubation comparable with the positive control. This fermentation manifests by the raise of dough in inoculated flasks by tested isolate and positive control but not with negative control (Figure 4). However, the S15P2 and S1P3 isolates do not fermented the paste even after 24 H incubation.

The pH was measured at the beginning and end of fermentation of the wheat flour dough. Figure 5 shows that the initial pH values of prepared pastes before fermentation are slightly acid. They range between 6.2 and 6.7. After a fermentation period of 5 Hours, the pH is remained slightly acid but it has decreased about 0.9 in the positive control while the reduction in tested isolates varied of 0.4 to 0.5 pH.

Table 6. Test of fermentation of the wheat flour paste by each yeast isolate

Isolate	S7P1	S14P1	S18P1	S14P2	S15P2	S20P2	S1P3	*Saccharomyces cerevisiae T*(+)
Fermentation ability	+	+	+	+	-	+	-	+

+ = raise the paste; **-** = no raise paste.

Figure 4. Fermentation tests of wheat flour dough by yeast isolates after 5 hours of incubation: a) From left to right: positive control, S18P1isolate, negative control; b) From left to right: positive control, S20P2 isolate, negative control

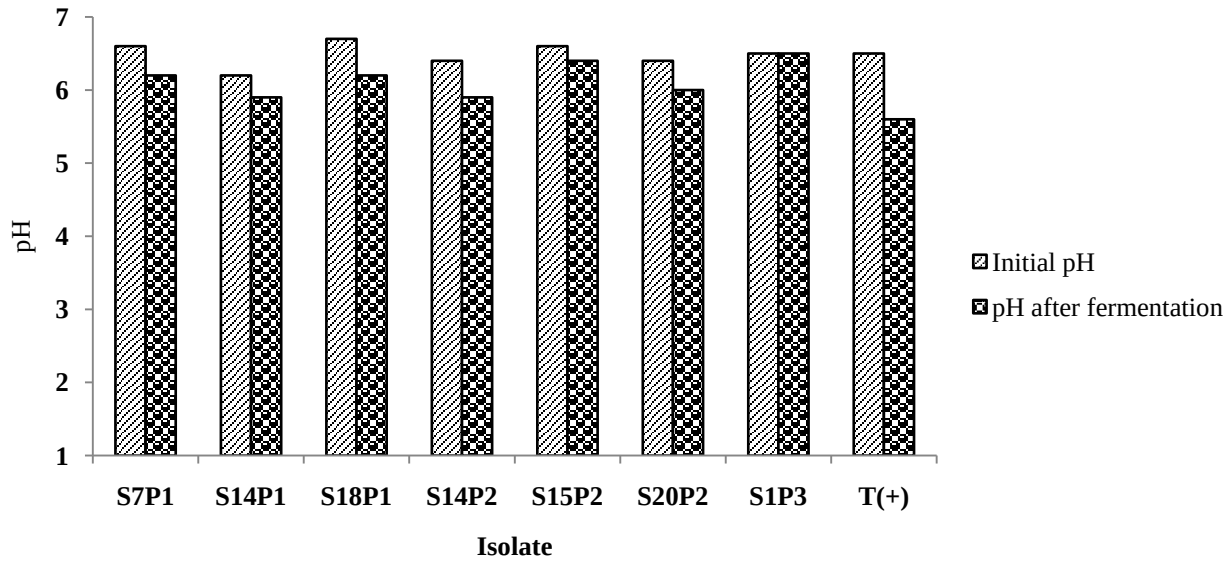

Figure 5. pH evolution at the beginning and the end of the dough fermentation (T(+) = positive control)

Table 7. Biochemical characterization of able yeast strains of raising wheat flour dough

Substrate	Strain					
	Control	S7P1	S14P1	S18P1	S20P2	S14P2
Glucose	+	+	+	+	+	+
Glycérol	-	-	-	-	-	-
2-Ceto-D-Gluconate	-	-	+	-	-	-
L-Arabinose	-	-	-	-	-	-
D-Xylose	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-
Xylitol	-	-	-	+	-	-
Galactose	+	+	-	-	+	+
Inositol	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-
α-Methyl-D-glucoside	+	-	-	-	-	-
N-Acetyl-D-Glucosamine	-	-	-	-	-	-
Cellobiose	-	-	-	-	-	-
Lactose	-	-	-	-	-	-
Maltose	+	+	+	+	-	-
Saccharose	+	+	+	+	+	+
Trehalose	-	-	-	-	-	-
Melezitose	-	-	-	-	-	-
Raffinose	+	+	+	+	+	+
Identification %	**99%**	**99,2%**	**57, 4%**	**89,5%**	**82.2%**	**82.2%**
Name Identified strain	***Saccharomyces cerevisiae***	***Saccharomyces cerevisiae***	**-**	***Rhodotorulamucilaginosa***	***Saccharomyces cerevisiae***	***Saccharomyces cerevisiae***

3.4. Identification of Yeast Isolates with API 20C AUX

The reactions of isolates five and positive control to 15 API 20 C AUX biochemical assays were identical (Table 7). For the four remaining biochemical assays the behavior was different. Indeed, the S10P2 and S14P2 isolates did not assimilate maltose (Mal); positive control, S14P1, S7P1 and S12P1 strains do not assimilate galactose (GAL); Only S14P1 used 2-ceto D gluconate (2KG) in its metabolism while S12P1 assimilated xylitol (XYL). Then the positive control strain was able to consume the α-methyl-D-glucoside substrate. Thus positive control, S7P1, S20P2 and S14P2 were identified with a percentage of 99%, 99.2%, 82.2% and 82.2%, respectively. These isolates were identified as *Saccharomyces cerevisiae* strains. The S14P1 isolates were identified with an identification percentage of 89.5% like *Rhodotorula g*enus. The S11P2 isolate wasn't identified to genus level.

3.5. Molecular Identification

In order to confirm strains identity obtained by biochemical methods, genomic DNA of 4 yeast strains S20P2, S7P1, S14P1, S18P1, and positive control were extracted and purified. The concentration of the DNA was estimated according to the size marker of the supplier. Figure 6 shows the results of the migration of the genomic DNA on 1% agarose gel after staining with bromide of ethidium. The genomic DNA bands obtained are above the marker of size of which the largest fragment is to 10.000 pb. Figure 7 shows the result of electrophoresis on 2% agarose gel of PCR products of five yeast strains obtained with species-specific primer pair to *Saccharomyces cerevisiae*. It reveals a single band of which the size corresponding at the specific gene to *S. cerevisiae* strain. This band appears on the track of S20P2, S7P1, S14P1, and S18P1strains and positive control. This result confirms the identity of these strains. On the other hand, the other oligonucleotide pairs don't give any band of which the size corresponding to specific genes of species *S. arboricolus, S. bayanus, S. kudriavzevii, S. Mikatae* and *S. paradoxus*.

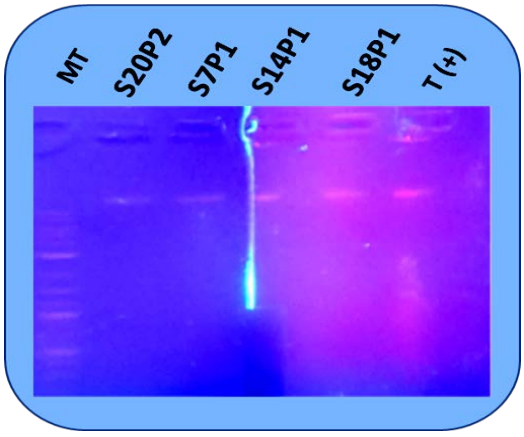

Figure 6. Gel agarose electrophoresis of genomic DNA of five yeast strains. **MT:** 2 Log ladder; S20P2: 20 strain isolated of 2 palm wine sample; S7P1: 7 strain isolated of 1 palm wine sample; S14P1: 14 strain isolated of 1 palm wine sample; S18P1: 18 strain isolated of 1 palm wine sample; T (+): positive control (baker's yeast)

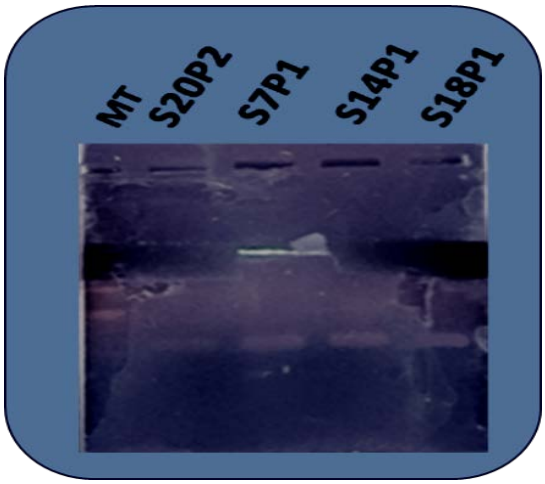

Figure 7. Gel agarose electrophoresis of PCR products of four yeast strains obtained with specific primer of *Saccharomyces cerevisiae* species. **MT:** 2 log ladder; S20P2: 20 strain isolated of 2 palm wine sample; S7P1: 7 strain isolated of 1 palm wine sample; S14P1: 14 strain isolated of 1 palm wine sample; S18P1: 18 strain isolated of 1 palm wine sample.

2.6. Effect of Temperature and pH on Yeast Growth

The temperature influence on the growth of four isolates raising the paste was studied at room temperature (between 25-28°C), 37°C, 44°C and 50°C. Figure 8 shows that all isolates grow at room temperature with an Optical Density (OD) slightly higher for the positive control (0.250). Nevertheless, S20P2 and S7P1 isolates have an OD higher than other isolates remaining 0.244 and 0.229, respectively. At 37°C, only growth of positive control has increased with OD of 0.281. On the other hand growth of S7P1, S20P2, S14P1, and S18P1isolates has slightly fallen of 0.208, 0.174, 0.196, and 0.192, respectively. At 44 °C and at 50°C growth of all isolates is very weak with OD which are less than or equal to 0.055.

Isolates were able to grow in the pH range of 5 to 9 (Figure 9). The growth of strains S14P1, S18P1, and T (+) increases as the pH increases (i.e., from acidity to basicity). S7P1 and S20P2 grow and reach their maximum growth at pH 7 and then decrease at pH 9. S18P1 has a maximum growth at pH 7, with O.D. value of 0.43 nm.

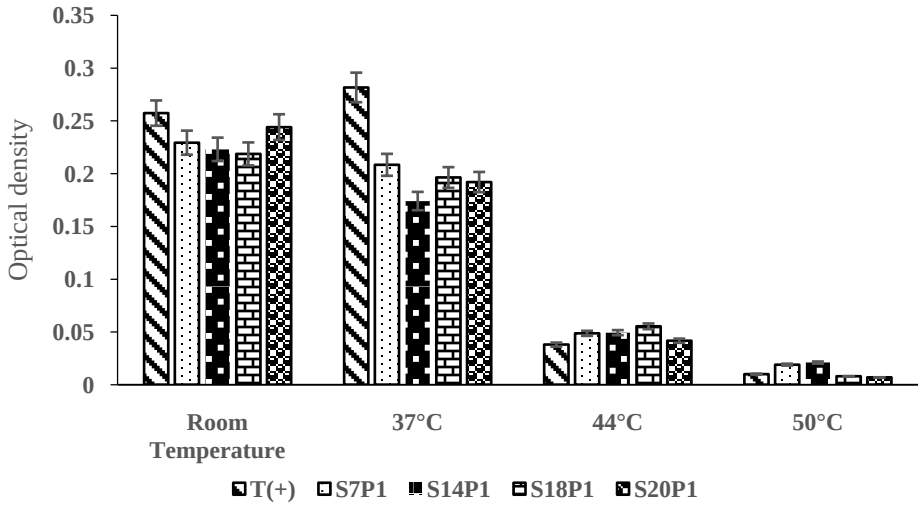

Figure 8. Effect of temperature on the isolates growth after an overnight incubation into liquid YPG medium.S20P1: 20 strain isolated of 2 palm wine sample; S7P1: 7 strain isolated of 1 palm wine sample; S14P1: 14 strain isolated of 1 palm wine sample; S18P1:18 strain isolated of 1 palm wine sample; T (+): positive control (baker's yeast)

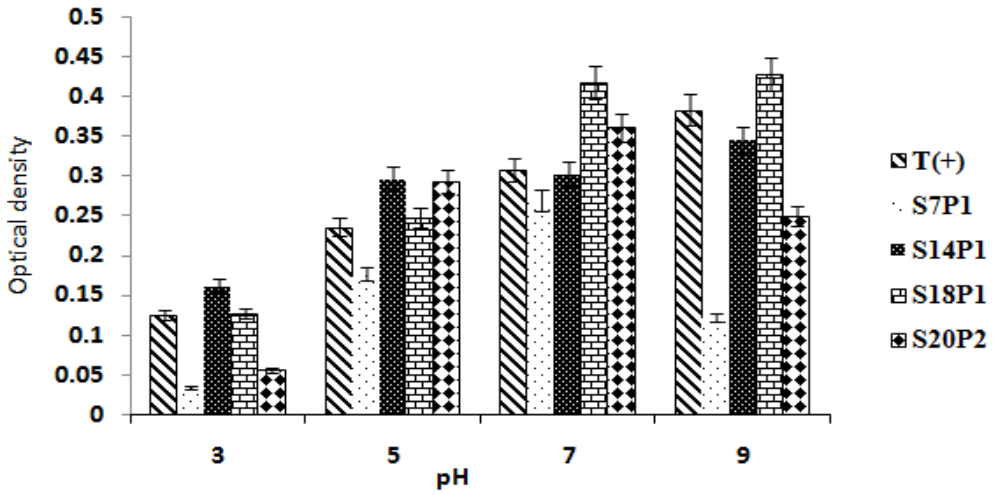

Figure 9. Effect of pH on the isolates growth in liquid YPG medium after an overnight incubation. S20P1: 20 strain isolated of 2 palm wine sample; S7P1: 7 strain isolated of 1 palm wine sample; S14P1: 14 strain isolated of 1 palm wine sample; S18P1:18 strain isolated of 1 palm wine sample; T (+): positive control (baker's yeast)

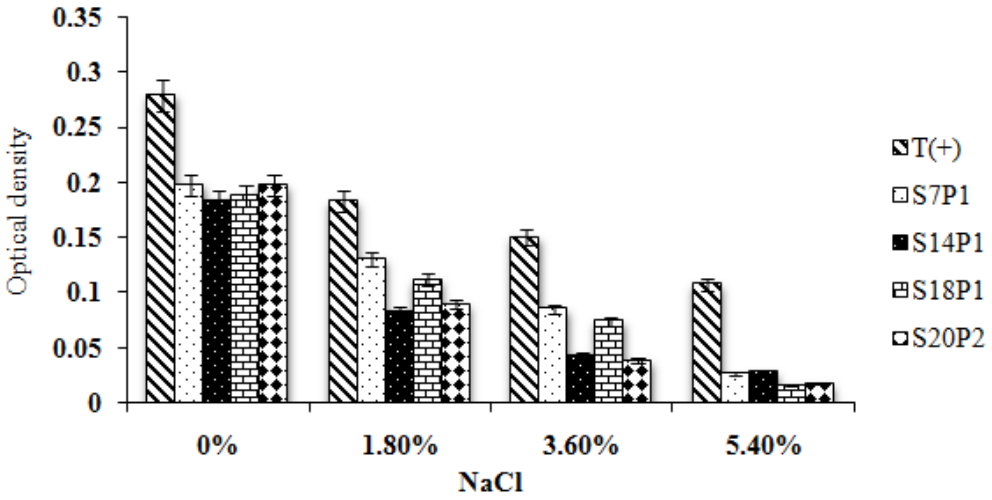

Figure 10. Effect of NaCl concentration on the isolates growth in liquid YPG medium after an overnight incubation.S20P1: 20 strain isolated of 2 palm wine sample; S7P1: 7 strain isolated of 1 palm wine sample; S14P1: 14 strain isolated of 1 palm wine sample; S18P1:18 strain isolated of 1 palm wine sample; T (+): positive control (baker's yeast)

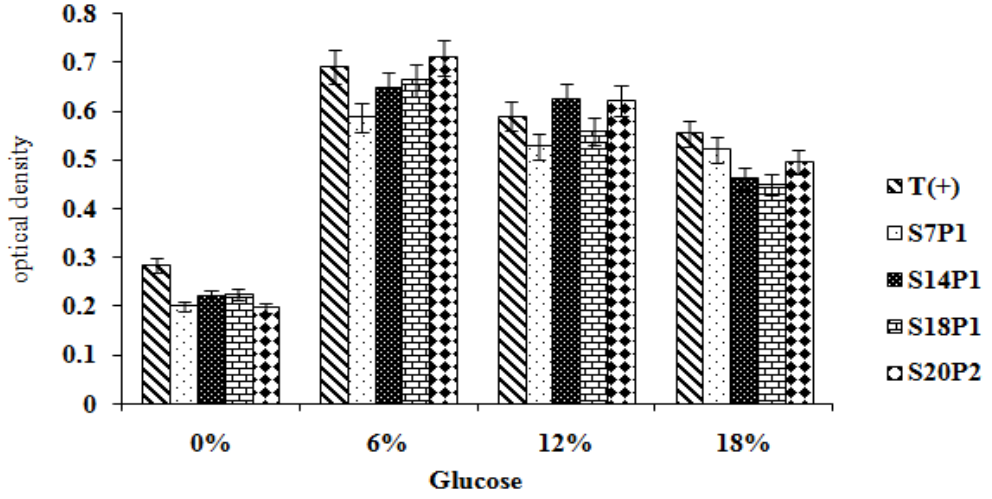

Figure 11. Effect of glucose concentration on the isolates growth in liquid YPG medium after an overnight incubation.S20P1: 20 strain isolated of 2 palm wine sample; S7P1: 7 strain isolated of 1 palm wine sample; S14P1: 14 strain isolated of 1 palm wine sample; S18P1:18 strain isolated of 1 palm wine sample; T (+): positive control (baker's yeast)

3.7. Effect of NaCl and Glucose on Yeast Growth

Growth of S14P1, S18P1, S7P1, S20P2 strains and T (+) decreases progressively as NaCl concentration increases (Figure 10). However, T (+) has maximum growth in medium without NaCl. Figure 11 shows the growth of four yeast strains and positive control in a range of glucose concentrations from 0% to 18%. In absence of glucose the yeast isolates and positive control growth was low. It increased at 6% with high O.D. for $S_{20}P2$, T (+), $S_{18}P1$, $S_{14}P1$ and S_7P1 at 0.71, 0.69, 0.66, 0.64 and 0.59, respectively. Then it decreased at 12%, with OD varying between 0.53 and 0.62. Maximum was obtained with S14P1 (0.62). This growth was continued to decrease at 18 % glucose but superior at the growth at 0% glucose.

4. Discussion

This study aims to isolate the palm wine yeasts with the ability to raise wheat flour dough. Yeasts enumeration showed that samples contain yeasts. Indeed, yeast number is between $0.3.10^6$ and 3.10^6 CFU/mL. Moreover, P3 sample presents higher yeast number while the number is weaker in the sample P2. These results seem to be in accordance previous works [7,15,16]. In these studies, the yeast analysis revealed total yeasts count in palm wine is included between 10^4 and 10^7 CFU/g. It is very important to highlight that results show also that yeast density depends on fermented food and its origin. It should be noted that yeasts constitute the significant micro-organisms during the first hours of the spontaneous fermentation of sap [17,18]. Fifty-one yeast isolates were obtained of three palm wine samples. However only 13 % isolates, S7P1, S14P1, $S_{18}P1$, $S_{14}P2$, $S_{15}P2$, $S_{20}P2$, and S1P have morphological characteristics close to *Saccharomyces* genus. These yeast isolates were tested for their carbon dioxide production ability. The gas production by yeasts isolates significantly remains weaker than the positive control represented by baker's yeast available in the local markets. Several authors [19,20] have showed that yeast isolates could utilized sugar different producing the acidic products and gas trapped in Durham tubes in YEG broth.

This production was higher than in our study. Indeed, the gas production weak could be proportional to the use of the poor medium in glucose.

Fermentation of wheat flour dough was highlighted by isolates capacity to raise the dough in the bottles after 5 hours of incubation. Tested yeast isolates do not ferment all with the same volume and for the majority to the 1/3 of the total volume of the bottles. Moreover, the fermentative potency of S_7P1, S_{18}P1, S_{14}P1, S_{14}P2, and S_{20}P2 yeast isolates dissimilar at the commercial yeast tested. In the previous works [21], it has been showed that wild yeasts isolated from palm wine are able to leaven the wheat flour dough. Other authors have been highlighted leavening dough capacity of yeasts isolated from other fermented foods [20,22,23]. This leavening dough capacity is related at the carbon dioxide release and the dough gluten. Indeed, two groups of proteins, prolamins and glutelins of wheat flour swell due to mechanical stress and atmospheric oxygen and they form a solid gel called gluten [24]. When yeasts release gas, this one is trapped in the closed network formed by the gluten that prevents the gas to escape. Thus, CO2 is attracted towards surface and exerts a pressure on network making inflate the dough.

The duration of 5h for our interest isolates allowed highlighting parameters like the acidity weak of the paste. According to a study carried out by Alais et al. [25], this period encompassing initiation phase of the biochemical reactions at the time of the metabolism, with the β-amylase which attacks starch granules damaged out of maltose and dextrin and α-amylase which cuts the starch chains in substrate for β-amylase. In order to highlight the fermentative process, dough pH was measurement. The results show also that the pH values ranging between 5.9 and 6.5 close to neutrality. In the same way Agro et al. [23] showed that the fermentation of rice flour dough the pH values ranging between 4.4 and 4.6. The Moroccan traditional leavens fermented the dough with the pH ranging between 3.0 and 3.7 [22]. The acidity of our dough samples could depend on the metabolic specificity of isolates. Our study appears particular interesting owing to the fact that the neutrality generated by these isolates would give a biotechnological advantage.

The identification of five isolates from palm wines and having fermented the wheat flour dough after 5hours only was carried out by API 20C AUX strip. The S14P2, S7P1, and S20P2 isolates were identified like *Saccharomyces cerevisiae* species, S14P1 like *Rhodotorula glutinis* and S18P1 like *Rhodotorula mucilaginosa*. The homology of genomic sequence of *Rhodotorula* spp. and *Saccharomyces cerevisiae* are close to 99%. Identification of the yeast using the API 20C AUX strip generally identified isolates to the genera level, and in other cases to the species level. These yeasts species were isolated in the studies of several authors [6,26,27]. It is important to worthy note that identifications-based API 20C AUX strip are often limited, so PCR amplification methods have been developed to characterize and distinguish Yeast isolates. PCR-based identification with specific oligonucleotide pairs of six species of *S. cerevisiae, S. mikatae, S. paradoxus, S. arboricolus, S. kudrieavzevii and S. bayanus* carried out in Muir et al. [14] studies confirmed strains performing in biochemical method including S20P2, S7P1 and S14P2 strains corresponding to *S. cerevisiae*. However, identification of S14P1 and S18P1 strains is contradictory compared to the biochemical identification carried out by the API 20 C AUX strip. Both strains were also identified as *S. cerevisiae*. These results are not surprising because the limits of identification by the API method were already documented [6,28,29,30,31]. Identification of microorganisms based only phenotyping is not reliable for some microorganisms. Thus, results obtained by molecular identification were retained.

Several physico-chemical parameters are important for the yeasts packaging and bread-making additives. The growth of all the strains fermenting paste at room temperature and at 37°C revealed that they are strains mesophilic like documented before. The temperature of proliferation of yeasts is included 12 and 40°C. A light growth for certain strains and an absence for others at 50°C were observed. What illustrates the absence of the thermophilic strains among these isolated yeasts [32].

All the strains having fermented the paste and identified grow with a going pH from 5 to 9 [18]. The best growth of our strains is observed with pH 7 which is favorable for the metabolic and enzymatic activities of the yeasts [32,33]. S7P1 strain has a growth around the concentrations of 2 % out of NaCl. A thorough study could be interesting to optimize the growth of the strains between 2 and 4 % out of NaCl within the framework of making bread and other derived products. The growth of all the strains having fermented the paste with the concentrations of glucose from 0% to 18% reveals that our strains tolerate a broad range of glucose. The yeasts have a tolerance of sugar concentration about 3M. It was showed that *Saccharomyces cerevisiae* strains have a good growth with the concentrations from 0% to 6% [13]. Our yeasts open a significant way in their use in the wine making, the brewery, in the pastry making and the manufacture of other products.

5. Conclusion

This study showed that palm wine used in the majority of the traditional ceremonies in Republic of Congo contains wild *Saccharomyces cerevisiae* strains with ability to raise wheat flour dough. It can be exploited as source of *Saccharomyces cerevisiae* which be used in industrial product of bio-leaven.

Conflict of Interest

The authors have no conflict of interest with regards to this publication.

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