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Research Paper

PHYSIOLOGICAL PROFILES OF INDIGENOUS YEASTS ISOLATED FROM RAFFIA WINE ORIGINATED OF CÔTE D'IVOIRE

Tiemele Laurent Simon Amoikon, Francine Moussan Aké, Mamadou Bakayoko, Théodore N'Dédé Djéni, Elisabeth Barbara Lidivine Mougo and Marcellin Koffi Djè

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.

Abstract

Yeasts are important agents in traditional beverages fermentation. Raffia wine is one of these beverages which indigenous yeasts are less investigated for their technological properties in food industry. The aim of the present study was to investigate some physiological traits of raffia wine main yeast species, in order to highlight their potential application areas. Among the 11 yeast species identified using sequencing of the LSU D1/D2 domain, 63.64% were thermo-tolerant and 36.36 % osmo-tolerant. *S. cerevisiae* ADR1 displayed the highest maximum growth rate (0,51 h^{-1}) and the lowest doubling time (1,36 h). Morever, all of them showed abilities to use D-glucose and *H. jakobsenii* ADR2 was the strain which assimilated the maximum of carbon source. These yeasts exhibited appreciable amino-peptidase properties and variable esterase, glycosyl-hydrolase and phosphatase activity as demonstrated by the API-ZYM test. This study represents a first steps of indigenous strains charaterization in order to promote technological properties of these yeasts for food applications.

Key words: Growth kinetic; thermo-tolerance; Osmo-tolerance; raffia wines; yeasts.

INTRODUCTION

Palm wine is a traditional beverage result to an uncontrolled fermentation involving indigenous microorganisms composed by yeasts, lactic acid bacteria and acetic acid bacteria [1]. Yeasts are one of the most important groups of palm wine microbiota. Their are responsible of alcoholic fermentation and also contribute to organoleptic caracteristics of this beverage [2]. Their presence in palm wine is related to raw

materials, equipment and local environments or from residues of previous fermentation batch [3]. This last decade, a growing interest have been devoted to the isolation and identification of yeasts species involving in fermentation process of palm wine. Santiago-Urbina *et al.* [4] showed that the composition of the yeast species in the samples varied among the different palm trees and also depend on environmental conditions and tapping. Most studies on palm wine have reported its potentials as source of yeast isolates for the fermentation [5]. In some traditional baker industries, palm wines are directly used as inoculum for leavening dough. Yeast strains contained in the palm sap are the leavening agents for dough [6]. Morever, Chilaka *et al.* [5] study has revealed that the use of palm wine yeasts to ferment passion fruit, water melon and pineapple could lead to acceptable wine. Also, this implies that palm wine could contain different and particular yeast species which might be use for technological applications. However, the use of pure strains in differents food industries requires knowledge of some physiologicals traits of these strains. Many studies were devoted to technological capacities of strains isolated from oil palm wine (*Elaeis guineensis*) but, very few papers deals with the technological abilities of raffia wine strains, although Ukwuru and Awah [7] demonstrated that raffia wine yeasts as oil palm wine yeasts had good properties for wine production from fruits. Thus, the purpose of this study was to investigate the physiological traits of raffia wine yeast strains in order to highlight some of their application areas.

MATERIALS AND METHODS

Samples, yeasts, chemicals and materials

Raffia wines were obtained from Côte d'Ivoire at Alepe (5° 30′ 2403″ N, 3° 39′ 53492″ W) and at Adzope villages (6° 6′ 25725″ N, 3° 52′ 19262″ W). Raffia wines were collected freshly from producers in the earlier hours of the morning (7:00 am) and were directly used to prepared ten-fold serial dilutions with sterile peptoned buffered water (BIO-RAD, France). About 100 µl of these dilutions were plated on YPDA (in w/v: 2% glucose, 1% yeast extract, 1% peptone and 2% agar) and plates were incubated at 30°C for up to five days. Representatives of each colony morphotype were then purified by repeated streaking on YPDA plates and identified to the species level by sequencing of the D1/D2 domains of the 26S rRNA gene, as previously described [8]. Table 1 presents the list of strains used in this study.

Temperature tolerance test

YPD liquid medium was used for determination of thermotolerance as described by Fakruddin *et al.* [9] with some modifications. Isolates were inoculated on YPD medium and the initial optical density of each tube was recorded on spectrophotometer at 600 nm against the medium as blank. Cultures were incubated at 25°C, 30°C, 35°C, 37°C, 40°C, 42°C and 45°C for 72 hours for observing thermo tolerance of yeast strain. The increase in optical density in a tube was recorded as evidence of growth. Tests were performed in triplicate.

50% glucose tolerance test

The test were performed according to wang *et al.* [10] with some modifications. All isolates were inoculated on medium containing 50% (w/v) glucose and incubated at 30°C for 2 weeks o screen for osmotolerant yeasts. The medium YPGA50 containing Yeast extract (1% w/v), Peptone (1% w/v), Glucose (50% w/v) and Agar (2% w/v) was used as selective medium. The tests are done in triplicate.

Determination of kinetic parameters

Kinetic parameters are evaluated as described by Alloue-Boraud *et al.* [11]. Cultures was realized on YPD medium. About 250 ml Erlenmeyer containing 50 ml of YPD were inoculated with cultures on solid medium and incubated for 24 h at 30 °C under agitation at 120 rpm. At regular times of two hours during the culture, samples were withdrawn for cell count and optic density measurements. According to Eqs. (1 and 2), kinetic parameters such as growth rate and doubling time were calculate.

$$\mu max = \frac{2.303(LogN0 - LogN1)}{(T1 - T0)} \quad (1)$$

$$g = \frac{Ln2}{\mu max} \quad (2)$$

μmax: maximal growth rate (h)

N_0 : cell concentration at the beginning of the exponential growth phase (CFU/ml)

T_0 : time of beginning of the exponential growth phase (h)

N_1 :cell concentration at the end of the exponential growth phase (CFU/ml)

T_1 : time at the end of the exponential growth phase (h)

g: generation time or doubling time (h).

Carbon source assimilation

The API ID 32C AUX (bioMérieux, France) systems were used for auxanogramm test. It's a commercial kit for the evaluation of the assimilation of selected multiple carbon sources. This test is conducted according to the manufacturer's instructions. The ampule of API C medium which was previousely inoculated with yeast strain culture was homogenized and 135 µl of the suspension was dispensed into each cupule of the strip. Then, the strip was incubated at 29° C + 2° C for 24-48hours

API-ZYM tests

Enzyme activities were evaluated with API-ZYM kits (API system, BioMerieux). Isolates were collected and suspended in sterile water and the suspension density was ajusted to 5 of McFarland scale. This suspension was used to inoculate the API-ZYM kits. The strips were incubated at 37°C for 4 h and reagents added according to the manufacturer's instructions. Visible changes in the color of the medium were considered positive. The intensity of the color reflected the concentration of the degraded substrate produce by the enzyme and enzyme activity was expressed in nmol of hydrolyzed substrate according to the scale provided in the kit: 1 corresponded to 5 nmol, 2 to 10, 3 to 20, 4 to 30, 5 to 40 and above. All tests were repeated 3 times for each strain [12].

Data analysis

Analysis of variance (Anova) and Tukey HSD tests were performed with XLSTAT software 2017 (Addinsoft Inc., Paris, France) to compare maximum growth and doubling time of yeast strains. Statistical differences with $p < 0.05$ were considered significant.

RESULTS

Yeasts strains were isolated in raffa wine from two sampling area (Adzope and Alepe) from Côte d'Ivoire. About 11 isolates of which 4 originated from Adzope wine and 7

from Alepe wine were identified by sequencing D1/D2 domain of LSU (Table 1). This strains were tested for etablishing their physiological profile.

Table 1 : List of studied strains

Strains	Sampling area	Accession number NCBI
Saccharomyces cerevisiae ADR1	Adzope	MG833305
Hanseniaspora jakobsenii ADR2	Adzope	MG833303
Geotrichum candidum ADR3	Adzope	MG833313
Yarrowia lipolytica ADR4	Adzope	MG833310
Kodamaea ohmeri ALR1	Alepe	-
Candida sorboxylosa ALR3	Alepe	-
Meyerozyma caribbica ALR4	Alepe	-
Yarrowia deformans ALR5	Alepe	-
Pichia manshurica ALR6	Alepe	-
Pichia kudriavzevii ALR7	Alepe	-
Debaryomyces hansenii ALR2	Alepe	-

Only strains which had not 100% of identity compared to their type strain was deposited in Genbank to obtained an accession number.

- : No accession number (strains match 100% to their type strain)

Temperature effet on yeast growth and growth in medium with 50% glucose

The yeast strains were tested for its growth at different temperature (Table 2). About 63.64% of the tested yeast strains tolerated temperatures up to 37°C. *C. sorboxylosa* ALR3, *M. caribbica* ALR4, *P. kudriazevii* ALR7, *P. manshurica* ALR6, *S. cerevisiae* ADR1 strains were able to grow at the maximal temperature of 42°C. Growth of yeasts on agar medium supplemented with 50% glucose was evaluated and the results were recorded in Table 1. Among the 11 strains, 4 (36.36 %) were able to grow on glucose 50% media. These are *D. hansenii* ALR2, *K. ohmeri* ALR1, *M. caribbica* ALR4, *P. kudriazevii* ALR7.

Table 2. Growth temperature evaluated on YPD medium and osmo-tolerance evaluated on medium supplemented with glucose 50% of raffia wine strains

Strains		Growth temperature							Growth in glucose 50%
		25°C	30°C	35°C	37°C	40°C	42°C	45°C	
Thermo sensitive	*D. hansenii* ALR2	+	+	+	+	-	-	-	+
	G. candidum ADR3	+	+	+	+	-	-	-	-
	K. ohmeri ALR1	+	+	+	+	-	-	-	+
	Y. lipolytica ADR4	+	+	+	+	-	-	-	-
Thermo tolerant	*Y. deformans* ALR5	+	+	+	+	+	-	-	-
	H. jakobsenii ADR2	+	+	+	+	+	-	-	-
	C. sorboxylosa ALR3	+	+	+	+	+	+	-	-
	M. caribbica ALR4	+	+	+	+	+	+	-	+
	P. kudriazevii ALR7	+	+	+	+	+	+	-	+
	P. manshurica ALR6	+	+	+	+	+	+	-	-
	S. cerevisiae ADR1	+	+	+	+	+	+	-	-

ALR : raffia wine from Alepe, ADR : raffia wine from Adzope

Maximum growth rate and doubling time

Kinetic parameters were determined from growth curve. This curve presented generally four periods such as latency phase, start-up phase, exponential phase and stationary phase. (Figure 1). According to maximum growth rate and doubling time, *S. cerevisiae* ADR1 growth started faster than the others studied strains. This strain growth phase lasts 8 hours and has the highest maximum growth rate (0.5109 h^{-1}) and the lowest doubling time (1.3568 h) (Table 3). Contrariwise, the strain *D. hansenii* ALR2 presented the slowest growth kinetic. The maximum growth rate of this strain was 0.1550 h^{-1} with a doubling time of 4.4708 h.

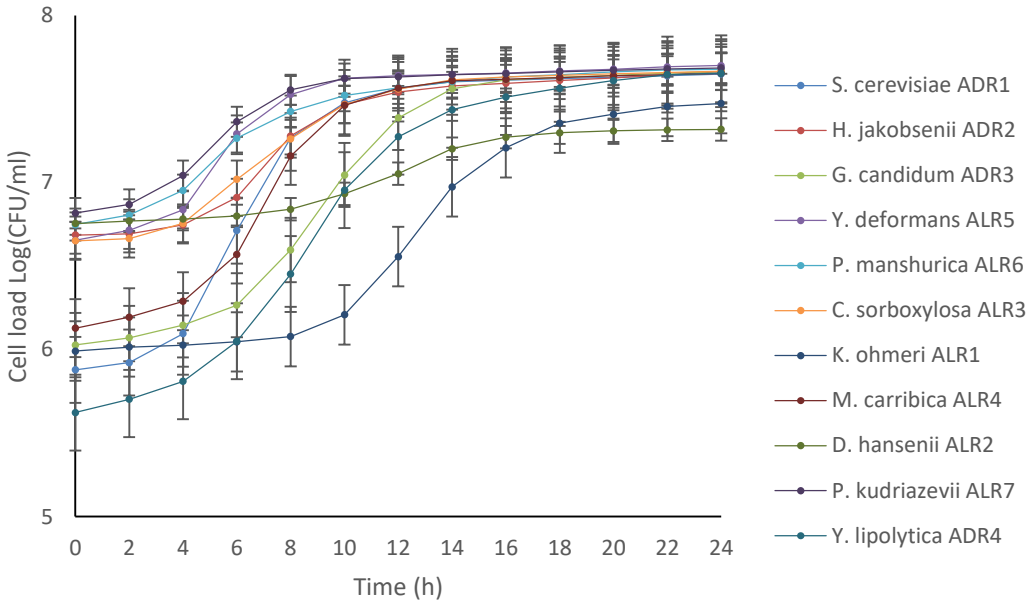


Figure 1. Kinetic curve of yeasts strains from raffia wine on YPD incubated for 24 h at 30 °C under agitation at 120 rpm.

Table 3. Maximum growth and doubling time of raffia wine isolates evaluated on YPD medium

Strains	Maximum growth rate (h^{-1})	Doubling time (h)
D. hansenii ALR2	0.1550 ± 0.0000^{a}	4.4708 ± 0.0005^{a}
P. manshurica ALR6	0.2184 ± 0.0000^{b}	3.1740 ± 0.0002^{b}
C. sorboxylosa ALR3	0.2313 ± 0.0000^{c}	2.9968 ± 0.0002^{c}
P. kudriavzevii ALR7	0.2951 ± 0.0000^{d}	2.3492 ± 0.0001^{d}
H. jakobsenii ADR2	0.3201 ± 0.0000^{e}	2.1656 ± 0.0002^{e}
K. ohmeri ALR1	0.3834 ± 0.0001^{f}	1.8077 ± 0.0005^{f}
Y. deformans ALR5	0.3976 ± 0.0000^{g}	1.7435 ± 0.0001^{g}
Y. lipolytica ADR4	0.4214 ± 0.0002^{h}	1.6448 ± 0.0008^{h}
G. candidum ADR3	0.4300 ± 0.0000^{i}	1.6119 ± 0.0002^{i}
M. caribbica ALR4	0.4501 ± 0.0000^{j}	1.5400 ± 0.0003^{j}
S. cerevisiae ADR1	0.5109 ± 0.0001^{k}	1.3568 ± 0.0004^{k}

The values are the means of three independent trials ± standard deviations. On the same column, mean values with the different letter are significantly different ($p > 0.05$).

Carbon sources utilization

All tested strains were able to assimilate D-glucose and 90.91% assimilated D-mannitol. Only *P. manshurica* ALR6 was not able to use D-mannitol (Table 4). Levulinic acid was not used by any strains of the study, whlie *H. jakobsenii* ADR2 was able to metabolize all the other sugars in the kit. On contrary, only two carbon sources (D-glucose and glucosamine) were assimilated by *P. manshurica* ALR6.

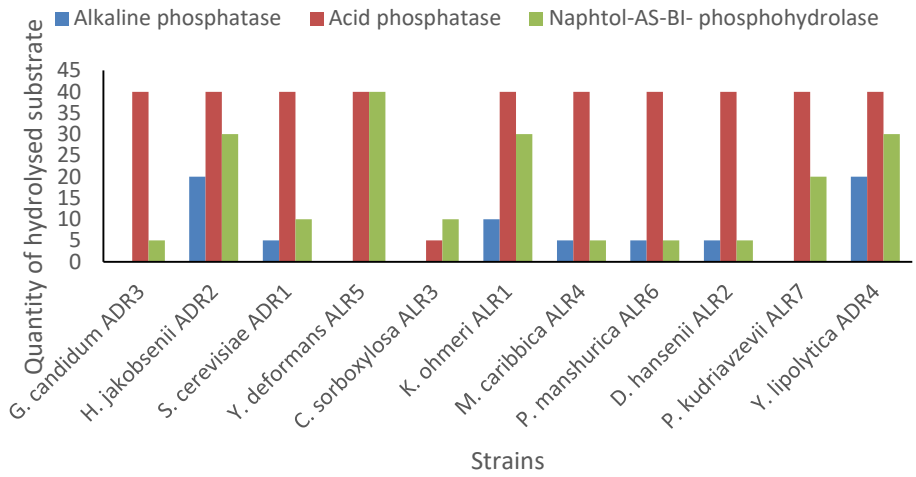
Table 4. Carbon sources assimilation by raffia wine yeasts determined with API ID 32 C AUX kits.

Carbon sources	*P. kudriazevii* ALR7	*M. carribica* ALR4	*D. hansenii* ALR2	*K. ohmeri* ALR1	*C. sorboxylosa ALR3*	*H. jakobsenii* ADR2	*P. manshurica* ALR6	*S. cerevisiae* ADR1	*Y. deformans* ALR5	*G. candidum* ADR3	*Y. lipolytica* ADR4	Frequency (%)
Galactose	+	+	+	+	+	+	-	+	-	+	+/-	81.82
Actidione	-	+	+	+	+	+	-	-	+	+	+	72.73
Saccharose	+	+	+	+	+/-	+	-	+	-	+/-	-	72.73
N-acetyl-glucosamine	+	+	+	+	+/-	+	-	-	-	+/-	-	63.64
Lactiv acide	-	-	-	-	+/-	+	-	+	-	+/-	-	36.36
L-arabinose	-	+	+	+	-	+	-	-	-	-	-	36.36
D-cellobiose	+	+	+	+	-	+	-	-	-	-	-	45.45
D-raffinose	+	+	+	+	-	+	-	+	-	-	-	54.55
D-maltose	+	+	+	+	+/-	+	-	+	-	+/-	-	72.73
D-trehalose	+	+	+	+	-	+/-	-	+	-	-	-	54.55
potassium 2-ketogluconate	+	+	+	+	-	+	-	+	-	-	-	54.55
Méthyl-αD-glucopyranoside	+	+	+	+	-	+	-	+	-	-	-	54.55
D-mannitol	+	+	+	+	+	+	-	+/-	+	+	+	90.91
D-lactose	-	-	-	-	-	+	-	-	-	-	-	9.91
Inositol	-	-	-	-	-	+	-	-	-	-	-	9.91
D-sorbitol	+	+	+	+	+	+	-	+	-	+	-	72.73
D-xylose	-	+	+	+	+	+	-	-	+/-	+	+/-	72.73
D-ribose	+	+	-	-	-	+	-	-	+/-	-	+	45.45
Glycerol	+	+	+	+	+	+	-	-	+	+	+	81.82
Rhamnose	-	+	-	-	-	+/-	-	-	-	-	-	18.18
Palatinose	+	+	+	+	+/-	+	-	+	-	+/-	-	72.73
Erythritol	-	-	+	+/-	-	+	-	-	+/-	-	+/-	45.45
D-melibiose	-	+/-	+	-	-	+	-	+	-	-	-	36.36
Sodium glucuronate	-	-	+	-	-	+	-	-	-	-	-	18.18
D-melezitose	-	+	+	+	-	+	-	-	-	-	-	36.36
Potassium gluconate	-	-	-	-	+/-	+	-	-	-	+/-	-	27.27
Levulinic acid	-	-	-	-	-	-	-	-	-	-	-	0
D-glucose	+	+	+	+	+	+	+	+	+	+	+	100
L-sorbose	+	+/-	+/-	+	+	+	-	-	-	+	-	63.64
Glucosamine	-	+	+	+	-	+	+	-	-	-	-	45.45
Esculin ferric citrate	+	+	+	+	+	+	-	-	-	+	-	63.64

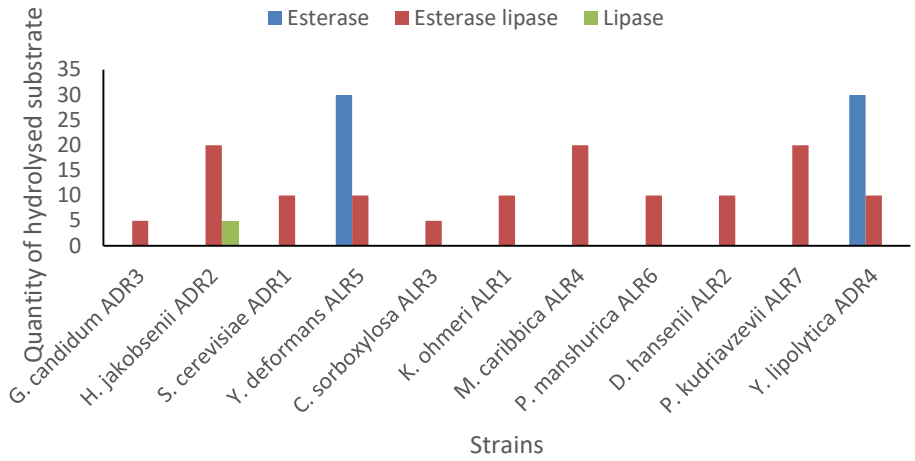
Enzymatic activities

Enzymatic profile of isolates were studied considering four enzymes families, notably esterase, phosphatase, amino-peptidase and glucosyl hydrolase. Almost all the strains displayed acid phosphatase and naphtol-ASBI-phosphohydrolase of at least 40 nanomoles of substrate hydrolyzed, excepted *Candida sorboxylosa* ALR3 (Figure 2a). They also displayed interesting esterase lipase activity, excepted *H. jakobsenii* ADR2 which lipase activity was low (5 nanomoles of substrates hydrolysed) (Figure 2b). Esterase was produced by *Yarrowia deformans* ALR5, *Yarrowia lipolytica* ADR4 at the same level (30 nanomoles). In the family of amino-peptidase, leucine and valine arylamidase were the common enzymes to all isolates. Isolates showed a higher activity of leucine arylamidase (40 nanomoles for all) but variable activity of valine arylamidase (Figure 2c). *H. jakobsenii* ADR2 had the highest valine arylamidase activity (40 nanomoles). Among the tested strains, 5 produced β-glucosidase, *Kodamaea ohmeri* ALR1 and *Yarrowia lipolytica* ADR4 displaying the highest activities (40 nanomoles). Four strains had α-glucosidase, with the best activity observed with*Meyerozyma caribbica* ALR4 (40 nanomoles).

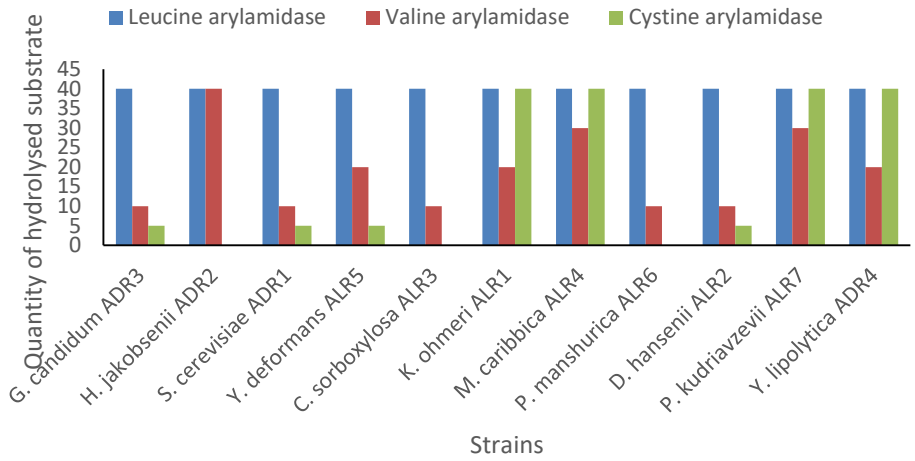
a) Phosphatase



b) Esterase



c) Amino-peptidase



d) Glycosyl-hydrolase

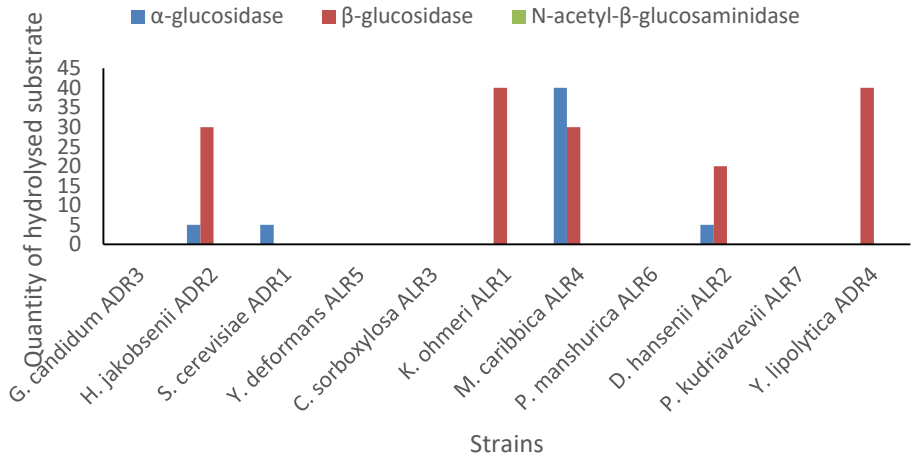


Figure 2. Enzymatic activity of raffia wine yeasts.determinated with API ZYM kits according to the manufacturer's instructions.

DISCUSSION

The use of yeasts in food industry depends on their physiological properties. Indeed, several conditions of environment influence yeast behaviors by affecting their growth and production capacities [13]. These conditions are temperature, oxidation, ethanol concentration or co-products in the culture medium, as well as the high concentration of sugars [14]. In this study, we focused on the effect of temperature and osmotolerance on the growth of indigenous yeasts isolated from raffia wine. The growth of some raffia wine isolates on medium supplemented with glucose 50% revealed the osmotolerant capacity of these yeasts. Osmotolerant yeasts are distinguished by their ability to survive at high osmotic pressure in environment. This capacity can be advantageous and economical in the biotechnological process of various foods and compounds. Indeed, in pastry, beyond 5%, the sugar exerts a pressure on plasma membrane. Yeasts need time to adapt to this pressure. These yeasts will take longer time to start fermentation and can even be inhibited by sugar-rich substrates. In contrast, so-called "osmotolerant" yeasts resist to pressure and are instantly active. The use of such yeasts in recipe "sweet brioche" is a real advantage. Also, in industries of confectionery, fruit juices and syrup, osmotolerant yeasts are widely used. *D. hansenii*, *K. ohmeri*, *M caribbica* and *P. kudriazevii* of our study survive at high concentration of sugars as previously reported by Mukherjee *et al.* [15]. These authors showed that *D. hansenii* and *K. ohmeri* are tolerant species at high glucose concentrations (≥ 50%) while *M. caribbica* and *P. kudriazevii* are tolerant to high glucose concentrations near 50%.

During this work, the effect of temperature on yeast growth was determined. Temperature is a factor that deeply affects microorganisms, like human. Some studied strains were predominant at maximal temperature of 37 °C but others predominated at temperature beyond 37 °C. Our results agree with those of Torija *et al*. [16] who reported that temperature affected the development of the indigenous yeast strains and influenced their metabolic pathways. In food industries, several processes require thermo-tolerant strains. Indeed, heat is released during fermentation due to exothermic reactions causing the increase of temperature (40 °C - 42 °C). In addition, thermotolerant strains are more suitable for use in tropical countries where the temperature is high. Thermo-tolerance of yeasts could be due to the metabolic changes within cells. Mensonides *et al.* [17] reported that upon heat stress, trehalose and

glycogen accumulate in the cell and trehalose contribute to the acquisition of thermo-tolerance.

The interest of the kinetic growth is to known the different phases and growth parameters of yeasts in non-renewed medium or Batch culture in order to optimized biomasse and metabolite production in food production processes. Latence phase, maximum growth and doubling time could be used to select appropriate fermentative yeast strains. In the particular case of *S. cerevisiae*, the maximum growth was reached after 8 hours following the lag phase. These results are identical to those of N'guessan [18] on pure cultures of *S. cerevisiae* isolated of tchapalo (sorghum beer). This phase is followed by a constant phase reflecting the depletion of glucose and amino acids in the culture medium. The doubling time of raffia wine yeasts varied between 1.3568 h (1 h 21 min 24 s) and 4.4708 h (4 h 28 min and 14 s). Several studies ([19], [20], [21]) noted that the kinetic parameters depend on the composition of the culture medium (nature and availability of the carbon substrate) and the culture conditions (temperature, renewal of the culture medium). The doubling time of *S. cerevisiae* on YPD which was 1.3568 h is similar with Alloue-Boraud *et al.* [11] findings, confirming that *S. cerevisiae* is an unicellular organism with a short generation doubling time 1.25 - 2 h at 30 °C and can be easily cultured. Species of the genus *Yarrowia* had doubling time evaluated to 1.6448 h and 1.7435 h for *Y. lipolytica* and *Y. deformans* respectively. These values are higher than those of Michely *et al.* [22] who found 1.56 h and 0.89 h for *Y. lipolytica* and *Y. deformans* respectively after growth on minimal base medium (MMB) supplemented with 0.15% yeast extract and 2% glucose.

Carbohydrate assimilation tests are useful in yeast diversity studies exploring new ecological niches. Carbohydrate uptake reactions are among the main tests used to differentiate between genera and yeast species, although they are limited in scale identification [23]. Also, knowledge of the assimilation profile of sugars by yeasts could allow to define the suitable substrates for these yeasts during the food production. All raffia strains have an affinity for glucose unlike other sugars where assimilation was variable for some strains. Flores *et al.* [24] explained this fact by the difference in the mechanisms of nutrients absorption, the number of isoenzymes and especially by the regulation of fermentation and respiration which differ significantly. Among carbohydrate uptake, some are fermented by yeasts strains to produce alcohol

primarily, others are not fermented but can contribute to the metabolism of yeast for the production of various secondary metabolites such as aroma compounds.

Raffia wine strains produced hydrolytic enzymes such as amino-peptidases, esterases, phospholipases and some glycosyl-hydrolase at different amounts. All the yeast strains showed appreciable amino-peptidase activity which was an important characteristic that have to be considered because amino-peptidase produce positive effects such as modifications of sensory quality (texture or flavor), enhanced digestibility, decreased allergenicity, or liberation of bioactive peptides [25]. Leucine arylamidase particularly was exibited by all isolates with a high activity. This enzyme is a good measure of the proteolytic activity of microrganisms. According to Flores *et al.* [24], leucine arylamidase catalyzes the cleavage of leucine and other hydrophobic amino acids from the amino terminus of protein or peptide substrates. The five strains *Debaryomyces hansenii* ALR2, *Hanseniaspora jakobsenii* ADR2, *Meyerozyma caribbica* ALR4, *Kodamaea ohmeri* ALR1 and *Yarrowia lipolytica* ADR4 were unique in producing β-glucosidase activity, an important property responsible for the hydrolysis of natural glucosides and influencing food flavour. The yeast strains tested displayed no other saccharolytic activity with the exception of α-glucosidase activity as reported by Psomas *et al.* [26].

CONCLUSION

Physiological profils of raffia wine strains were variable on the basis of parameters analysed in this study. Osmo-tolerant at 30 °C and thermo-tolerant yeasts strains were distiguished. Studied yeasts had different kinetic parameters and variable carbohydrate uptake. They also showed desirable enzymatic activities and could be used as supplements in foods. This research represents a preliminary study of physiological characterization of indigenous yeasts for foods applications but, to use them in food industry, these strains would undoubtedly require further investigations such as study of safe caracteristics, fermentation capacities, others enzymes activities, flavor enhancers capacities. However, the results obtained up to now allow to highlight the technological traits of each strains which may be use in food application according to production process involved.

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REFERENCES

[1] Amoa-Awua,W.K., Sampson, E., and Tano-Debrah, K., 2007, Growth of yeasts, lactic and acetic acid bacteria in palm wine during tapping and fermentation from felled oil palm (*Elaeis guineensis*) in Ghana, *Journal of Applied Microbiology*, vol. 102, pp 599-606.

[2] Noll, R.G., 2008, The wines of West Africa: history, technology and tasting notes, *The Journal of Wine Economics,* vol. 3, pp 85-94.

[3] Ogbulie, T.E., Ogbulie, J.N., Njoku, M.O., 2007, Comparative study on the shelf life stability of palm wine from *Elaeis guineensis* and *Raphia hookeri* obtained from Okigure, Nigeria, *African Journal of Biotechnology*, vol. 6, no. 7, pp 914-922.

[4] 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 south-east of Mexico, *Annals of Microbiology*, vol. 65, pp 287-296.

[5] Chilaka, C.A., Uchechukwu, N., Obidiegwu, J.E., Akpor, D.B., 2010, Evaluation of the efficiency of yeast isolates from palm wine in diverse fruit wine production, *African Journal of Food Sciences*, vol. 4, no. 12, pp 764-774.

[6] Olowonibi, O.O., 2017, Isolation and characterization of palm wine strains of *Saccharomyces cerevisiae* potentially useful as bakery yeasts, *European Journal of Experimental Biology*, vol. 7, no. 2, pp 11-24.

[7] Ukwuru, U.M., Awah, I.J., 2013, Properties of palm wine yeasts and its performance in wine making, *African Journal of Biotechnology*, vol. 12, no. 19, pp 2670-2677.

[8] Monnet, C., Straub, C., Castellote, J., Onesime, D., Bonnarme, P., Irlinger, F., 2013, Quantification of yeast and bacterial gene transcripts in retail cheeses by reverse transcription-quantitative PCR, *Applied and Environmental Microbiology*, vol. 79, no. 2, pp 469-477.

[9] Fakruddin, M., Islam, M.A., Quayum, M.A., Ahmed, M.M., Chowdhury, N., 2013, Characterization of stress tolerant high potential ethanol producing yeast from agro-industrial waste, *American Journal of BioScience.* Vol. 1, no. 2, pp 24-34.

[10] Wang, H., Zhongqiu, H., Long, F., Niu, C., Yuan, Y., Yue, T., 2015, Characterization of osmotolerantyeasts and yeast like molds from apple orchards and apple juice processing plants in China and investigation of their spoilage potential, *Journal of Food Science*, vol. 80, pp 1850-1860.

[11] Alloue-Boraud, W.A., N'Guessan, K.F., Djeni, N.T., Hiligsmann, S., Djè, K.M., Delvigne, F., 2015, Fermentation profile of *Saccharomyces cerevisiae* and *Candida tropicalis* as starter cultures on barley malt medium, *Journal of Food Science and Technology,* vol. 52, pp 5236-5242.

[12] Kurnatowski, P., Wójcik, A., Blaszkowska, J., Góralska, K., 2016, The hydrolytic enzymes produced by fungi strains isolated from the sand and soil of recreational areas, *Annals of Parasitology*, vol. 62, no. 3, pp 201-208.

[13] Li, S., Yang, X., Yang, S., Zhu, M., Wang, X., 2012, Technology prospecting on enzymes: application, marketing and engineering," *Computational and Structural Biotechnology Journal*, vol. 2, pp 1-11.

[14] Belloch, C., Orlic, S., Barrio, E., Querol, A., 2008, Fermentative stress adaptation of hybrids within *Saccharomyces* sensu strico complex, *International Journal of Food Microbiology*, vol. 122, no. 1, pp 188-195.

[15] Mukherjee, V., Radecka, D., Aerts, G., Verstrepen, K.J., Lievens, B., Thevelein, J.M., 2017, Phenotypic landscape of non-conventional yeast species for different stress tolerance traits desirable in bioethanol fermentation, *Biotechnology for Biofuels*, vol. 10, pp 216-235.

[16] Torija, M.J., Rozes, N., Poblet, M., Guillamon, J.M., Mas, A., 2002, Effects of fermentation temperature on the strain population of *Saccharomyces cerevisiae, International Journal of Food Microbiology,* vol. 80, pp 47-53.

[17] Mensonides, F.I., Schuurmans, J.M., Teixeira de Mattos, M.J., Hellingwerf, K.J., Brul, S., 2000, The metabolic response of *Saccharomyces cerevisiae* to continuous heat stress, *Molecular Biology Reports*, vol. 29, pp 103-106.

[18] N'guessan, K.F., 2009, Analyse de quelques paramètres de la fermentation alcoolique conduisant au tchapalo (bière traditionnelle à base de sorgho) et des

potentialités à la formulation d'une culture starter à partir de levures isolées, Thèse de Doctorat Unique, Université Abobo-Adjamé. 216 p, 2009.

[19] Egli, T., 2015, Microbial growth and physiology: a call for better craftsmanship, *Frontiers in Microbiology*, vol. 6, pp 287-299.

[20] Füchslin, H.P., Schneider, C., Egli, T., 2012, In glucose-limited continuous culture the minimum substrate concentration for growth, S_{min}, is crucial in the competition between the enterobacterium *Escherichia coli* and *Chelatobacter heintzii*, an environmentally abundant bacterium, *International Society for Microbial Ecology*, vol. 6, pp 777-789.

[21] Kovárová-Kovar, K., Egli, T., 1998, Growth kinetics of suspended microbial cells: from single substrate-controlled growth to mixed-substrate kinetics, *Microbiology and molecular biology reviews*, vol. 62, no. 3, pp 646-666, 1998.

[22] Michely, S., Gaillardin, C., Nicaud, J-M., Neuvéglise, C., 2013, Comparative Physiology of Oleaginous Species from the *Yarrowia* Clade, *PLoS ONE*, vol. 8, no. 5, e63356.

[23] Devadas, S.M., Ballal, m., Prakash, P.Y., Hande, M.H., Bhat, G.V., Mohandas, V., 2017, Auxanographic carbohydrate assimilation method for large scale yeast identification, *Journal of Clinical and Diagnostic Research*, vol. 11, no. 4, pp 1-3.

[24] Flores, C.L., Rodriguez, C., Petit, T., Gancedo, C., 2000, Carbohydrate and energy-yielding metabolism in non-conventional yeasts, *FEMS Microbioly Review*, vol. 24, pp 507-529.

[25] Tavano, O.L., Berenguer-Murcia, A., Secundo, F., Fernandez-Lafuente, R., 2018, Biotechnological applications of proteases in food technology, *Comprehensive reviews in food science and food safety*, vol. 17, pp 412-436.

[26] Psomas, E., Andrighetto, C., Litopoulou-Tzanetaki, E., Lombardi, A., Tzanetakis, N., 2001, Some probiotic properties of yeast isolates from infant faeces and Feta cheese, *International Journal of Food Microbiology*, vol. 69, pp 125-133.