Scientific Bulletin. Series F. Biotechnologies, Vol. XXVII, No. 1, 2023
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

BIOETHANOL PRODUCTION THROUGH *Saccharomyces* AND Non-*Saccharomyces* YEAST STRAINS ISOLATED FROM COCOA MUCILAGE JUICE FERMENTED

Wahauwouele Hermann COULIBALY[1], Tano Marie-Ange Sakia MIAN[2, 3], Fatoumata CAMARA[2], Grah Avit Maxwell BEUGRÉ[3], Camelia Filofteia DIGUȚĂ[4], Florentina MATEI[4]

[1]Biotechnology and Food Microbiology Laboratory, Food Science and Technology, Training and Research Unit, University Nangui Abrogoua, Abidjan 02 BP 801, Ivory Coast
[2]Nutrition and Food Security Laboratory, Food Science and Technology, Trainning and Research Unit, University Nangui Abrogoua, Abidjan 02 BP 801, Ivory Coast
[3]Agro-Valorization Laboratory, Agroforestery Trainning and Research Unit, Jean Lorougnon Guédé University, Daloa, BP-150, Ivory Coast
[4]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding authors emails: wahauwouele@yahoo.fr, camelia.diguta@biotehnologii.usamv.ro

Abstract

Due to its significant contribution to lowering the use of crude oil and environmental pollution, bioethanol has been selected as the biofuel that is used the most frequently worldwide. The bioethanol was produced by fermentation carried out by microorganisms, particularly yeasts. Thus, yeasts resistance to ethanol remained a criterion important in bioethanol production. In this context, this study, the ability of Saccharomyces strain (Saccharomyces cerevisiae) and non-Saccharomyces strains (Debaryomyces hansenii, Rhodotorula mucilaginosa, and Pichia kudriavzevii) to produce bioethanol by sorghum wort fermentation following distillation was investigated. The results revealed that, globally, all yeast strains studied exhibited a similar fermentation behavior. The difference between yeast strains was observed in the alcoholic degrees of distillates. The alcoholic degree of distillates ranged between 7.01±0.007 and 7.38±0.063% (v/v) where the highest concentration has been observed with Rhodotorula mucilaginosa. More, statistical analysis showed a significant difference ($P<0.05$) between Rhodotorula mucilaginosa and other species and mixed culture. Among the strains studied, Rhodotorula mucilaginosa specie seemed the suitable strain to produce bioethanol.

***Key words**: bioethanol, fermentation, non-Saccharomyces strains, Rhodotorula mucilaginosa, Saccharomyces strains*

INTRODUCTION

Environmental disasters (e.g., pollution, global warming) caused by the use of fossil fuels have resulted in the development of new, more environmentally friendly, and less energy-intensive sources (Hoekman, 2009; Demirbas, 2010; Kiran et al., 2014). Thus, bioethanol known as ethyl alcohol or chemically C_2H_5OH or EtOH remains one of the oldest environmentally friendly resources in use. Although its use and production are essentially non-existent in Africa, this is not the case everywhere else. The first commercial uses of bioethanol were initiated in Brazil in 1925. At the beginning of the 20th century, bioethanol was widely used in European countries and the United States. The growing interest in bioethanol and its adoption as an alternative by several countries was observed in the early 1980s (Azhar et al., 2017). Depending on the raw materials used, there are three generations of bioethanol. Feedstocks high in sucrose (such as sugar cane, sugar beet, sweet sorghum, and fruits) and starch (corn, wheat, rice, potato, cassava, sweet potato, and barley) were used in the production of first-generation bioethanol. Wood, straw, and grasses were examples of lignocellulosic biomass that is used to produce second-generation bioethanol. Algal biomass, which includes both microalgae and macroalgae, has been used to produce third-generation bioethanol (Nigam et al., 2011). Also food industrial waste has been reported such as fermentation medium to produce high concentration of biotehanol (Gropoşilă-Constantinescu et al., 2019). Whatever the bioethanol generation, it is produced through

the fermentation process by microorganisms. On other hand, some technical regarding microorganisms are required to optimize bioethanol production such as use of powder inoculum and microorganism encapsulation (Safitri et al., 2017).

S. cerevisiae has long been known for its fermentation performance and high resistance to ethanol and has been widely used for bioethanol production (Choi et al., 2010; Zhao et al., 2010; Mussato et al., 2012; Scordia et al., 2012; Kumari et al., 2013; Kim et al., 2014; Mossi et al., 2018). However, other authors reported the use of non-*Saccharomyces* yeast species in bioethanol production, such as *Debaryomyces hansenii* (Calahorra et al., 2009; Kurian et al., 2014); *Rhodotorula mucilaginosa* (Bura et al., 2012), and *Pichia kudriavzevii* (Ndubuisi et al., 2018; Akita et al., 2021; Pongcharoen, 2022). For bioethanol production, the microorganisms involved must require some skills namely resistance to ethanol. Although there are no universal methods for determining the resistance of microorganisms to ethanol, studies have relied on the relative values of cell growth, the specific rate of ethanol production, cell viability, and proton flux across the plasma membrane (Thomas et al., 1979; Beavan et al., 1982; Jiménez et al., 1985; Dombek et al., 1986; Birch et al., 2000). Also, bioethanol production is one most important biotechnological properties of yeasts. Finding yeast strains that can produce bioethanol is always a current concern. Thus, in this study, the ability of *Saccharomyces* and non-*Saccharomyces* yeast strains isolated and identified from cocoa mucilage juice fermented to produce bioethanol by using sorghum wort was investigated.

MATERIALS AND METHODS

Yeast strains

One *Saccharomyces cerevisiae* strain (YA5) and 3 three non-*Saccharomyces* strains (AK2; AK3; TIAS6) isolated from the cocoa juice in fermentation where the ethanol rate ranged between 7 and 10% have been taken into account in this study. All strains belonged to culture collection of the Food Technology Department (University Nangui Abrogoua, Abidjan, Côte d'Ivoire) and maintained in a 30% glycerol solution, at -20°C.

These yeast strains have been identified by Polymerase Chain Reaction Restriction Fragment Length polymorphism (PCR-RFLP) of the Internal Transcribed Spacer (ITS) region and sequencing of D1/D2 domains of rDNA sequence. The codes of strains are following : *Saccharomyces cerevisiae* (YA5), *Rhodotorula mucilaginosa* (AK2), *Debaromyces hansenii* (AK3), *Pichia kudriavzevii* (TIAS6). They were used for carrying out the alcoholic fermentation.

Fermentation conditions

Triplicate fermentations were performed with agitation in 1-L sterile Erlenmeyer flasks that contained 500 mL of pasteurised sorghum wort (10 min at 100°C) and were sealed with dense cotton plugs. A dense suspension of each specie from a YPD agar containing 10 g/L yeasts extract (Difco); 10 g/L Bacto Peptone (Becton Dickinson); 10 g/L D-glucose (Sordalab), and 10 g/L Agar (Oxford) plate was prepared in sorghum wort using a loop. The flasks containing 500 mL sterile wort were inoculated with each specie in mixed and monoculture [(*S. cerevisiae* (YA5), *R. mucilaginosa* (AK2), *D. hansenii* (AK3), *P. kudriavzevii* (TIAS6)] at O.D. 0.5 (10^7 CFU/mL) and shaken at 120 rpm for 120 h at 25 °C. Over time, samples were collected each 24 h for physicochemical and microbiology analyses. Three independent experiments were carried out.

Analytical determination

pH, titratable acidity, and total soluble solids (TSS)

After calibration with phosphate buffer, the pH of the yeast cultures during fermentation was measured using a pH meter (Hanna Instruments; HI 8010). Titration with 0.1 N NaOH was used to evaluate the titratable acidity which is expressed in lactic acid meq%. Using a hand refractometer, the total soluble solids (TSS) concentration, reported as °Brix, was determined in each sample. This latter parameter has been used to determine the sugar consumption rate. Three separate measurements were taken for every parameter.

Gas (CO_2) production during alcoholic fermentation

An experimental technique described by Lai (2010) was used in combination with fermentation tests to measure the volume of gas produced during fermentation. The fermentations performed through of were carried out in 500 mL flasks Erlenmeyer. Samples are taken regularly during alcoholic fermentation. The flasks Erlenmeyer are weighed before Weight (t-1) and after Weight (t) each sample to determine the kinetics of CO_2 production.

$$CO_2 = CO_2\,(t-1)\,\frac{\text{Weight}\,(t-1) - \text{Weight}\,(t)}{\text{Volume}(t)}$$

Volume (t) = V0-n*Vp,
where:
V0 - Volume at t = 0
N - Number of sampling
Vp - Volume of the collected sample

Microbial growth during fermentation

The method mentioned by Antunovics et al. (2005) was used to cultivate the yeast during fermentation. The increase in cell population was observed using the optical density at 600 nm. The experiments were replicated three times.

Distillation of fermented sorghum worts

The fermented worts are distilled to extract ethanol using the vigorous column distiller Quickfit/FC3/13, which measures 85 cm in length and 4.45 cm in diameter. Until all of the alcohol in the fermented must be used up in the heating flask, the temperature at the head of the column was kept at 79°C. The ethanol content was determined using an alcohol meter. Three independent experiments were carried out.

Statistical analysis

The collected data were processed using statistical analysis. XLStat software was used to do an analysis of variance (ANOVA) (version 2016). Duncan and Tukey's tests were used to assess the mean values of the physicochemical parameters of fermenting worts and fermented worts. Values of $P < 0.05$ were regarded as significant differences.

RESULTS AND DISCUSSIONS

The role of yeast involved in bioethanol and beverage production still one of the most studied biotechnological properties (Novidzro et al., 2013; Alexandre, 2014; Gbohaida et al., 2016) in contrast to their role in biofuel production (Hadiyanto et al., 2013; Tofighi et al., 2014; Mardawati et al., 2022; Saleh et al., 2022). Thus, pH and titratable acidity changes during alcoholic fermentation were shown in Figure 1.

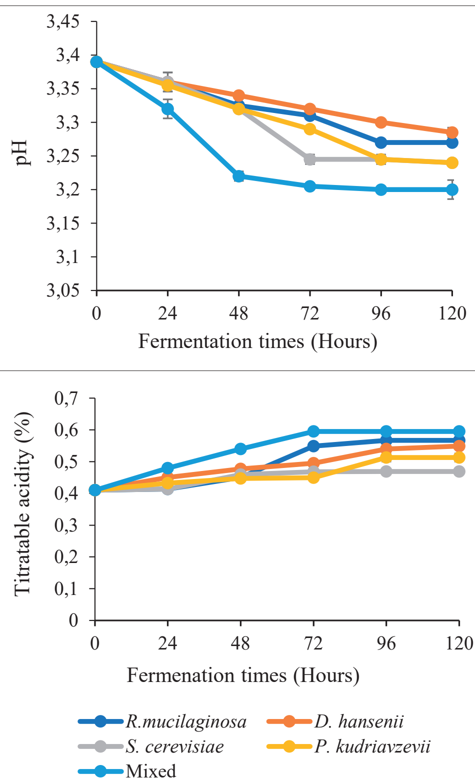

Figure 1. Changes of pH (a) and TA (b) during alcoholic fermentation of sorghum wort

During fermentation, it developed an inverse relationship between the sorghum worts fermenting pH and titratable acidity values (Figure 1). The change of the pH values was characterized by a decrease, while that of the titratable acidity increased. Thus, the mixed culture had more acidified the sorghum wort which expressed by more low value of pH 3.2 and more high value of 0.594% at the end of fermentation.

The growth performance of the yeast strains was assessed through the sugar consumption rate and the release of CO_2 during fermentation.

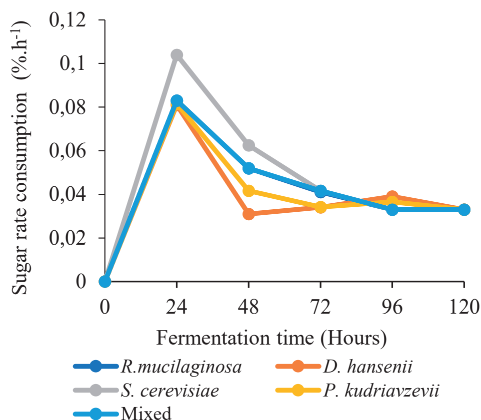

Figure 2. Sugar rate consumption during fermentation

Figure 2 showed the rate of sugar consumption by the yeast species used in this study during alcoholic fermentation. Regardless of the strain, an increase in the consumption rate was observed during the first 24 hours of fermentation, with the highest rate recorded for *S. cerevisiae* (0.104%.h^{-1}), while the rates for the other species were almost similar. After more than 24 hours, a decrease in the rate of sugar consumption was observed for the mixed culture and the *S. cerevisiae* and *R. mucilaginosa* species, from 0.083 to 0.033%.h^{-1} for the mixed culture and *R. mucilaginosa* species, respectively, after 96 hours, and from 0.104 to 0.033%.h^{-1} for the *S. cerevisiae* species after 96 hours of fermentation. From 96 h to the end of fermentation (120 h), the consumption rate remained constant at 0.033%.h^{-1}. The species *P. kurdiavzevii* was characterized by a decrease in the rate of sugar consumption from 24 h to 72 h, from 0.081 to 0.034%.h^{-1}, before a slight increase in the rate of consumption after 96 h, at 0.036%.h^{-1}. The *D. hansenii* species showed a decrease in the rate of sugar consumption from 24 to 48 h, with a slight increase in this rate between 48 and 96 h, from 0.031 to 0.039%.h^{-1}. However, at the end of fermentation (120 h), all species showed the same sugar consumption rate of 0.033%.h^{-1}. At the end of fermentation, no significant difference ($P>0.05$) was observed between the other samples.

CO_2 production during alcoholic fermentation by yeast species tested was presented in Figure 3.

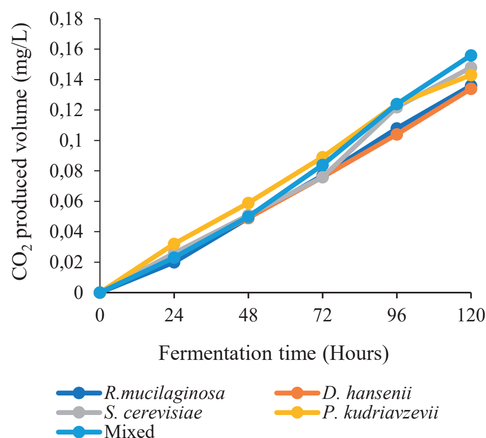

Figure 3. CO_2 produced volume (mg/L) during alcoholic fermentation

In general, a continuous increase in the volume of CO_2 released was recorded for all the yeast species tested, as well as the mixed culture. During the first 72 h of fermentation, the highest volume of CO_2 released was observed in the sorghum wort fermented by the *P. kurdiavzevii* species, increasing from 0 to 0.089 g/L. From 72 h to the end of fermentation, a more rapid increase in the volume of CO_2 released was obtained in the sorghum wort inoculated with *S. cerevisiae* species and the mixed culture. At the end of fermentation, the values for the volume of CO_2 released were 0.156 g/L for the mixed culture, 0.148 g/L for the *S. cerevisiae* species, and 0.143 g/L for the *P. kurdiavzevii* species. No significant difference ($P>0.05$) was observed between the other samples at the end of fermentation.

Yeast growth was determined by measuring its optical density during fermentation. Although this method is not precise enough, it does indicate the growth of the species tested. Thus, for all species tested, a continuous increase in OD values was recorded (Figure 4).

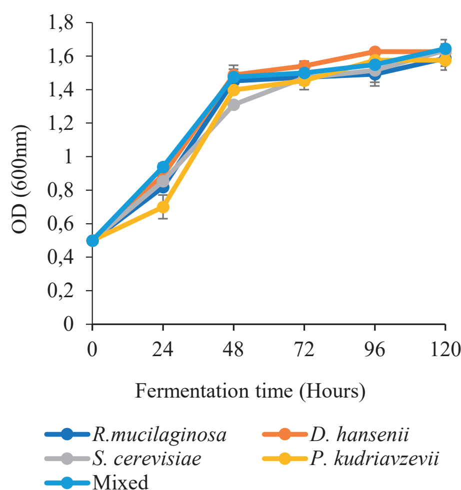

Figure 4. Yeast strains growth during alcoholic fermentation

For all the species tested, two growth phases were observed: a rapid growth phase during the first 48 hours with OD values increasing from 0.5 for all species and the mixed culture at the beginning of fermentation to 1.451; 1.487; 1.310; 1.470; 1.398; and 1.474, respectively, for the species *Rhodotorula mucilaginosa, Debaromyces hansenii, Saccharomyces cerevisiae, Pichia kurdiavzevii*, and the mixed culture. The second phase of yeast growth, which extends from 48 hours to the end of fermentation, was characterized by slower growth. The OD values evolved from 1.451 to 1.578 for *Rhodotorula mucilaginosa*; from 1.487 to 1.626 for *Debaromyces hansenii*; from 1.310 to 1.634 for *Saccharomyces cerevisiae* species; from 1.398 to 1.575 for *Pichia kurdiavzevii* cod; and from 1.474 to 1.644 for the mixed culture. Thus, this increase in OD correlated with the decrease in attenuation values and the increase in CO_2 release values. No significant difference ($P>0.05$) was observed between the other samples.

Overall, every strain exhibited remarkable fermentation performance, as seen by a continuous increase in CO_2 volume and optical density values as well as nearly comparable sugar consumption kinetics. The same characteristics have been reported by Mossi et al. (2018) and Coulibaly et al. (2021). This similarity between the different strains was also reflected in the alcohol content produced, which ranged from 7.01±0.007 to 7.12±0.084% (v/v) except *Rhodotorula mucilaginosa* where the alcohol content of distillate was 7.38±0.063% (v/v) (Figure 5).

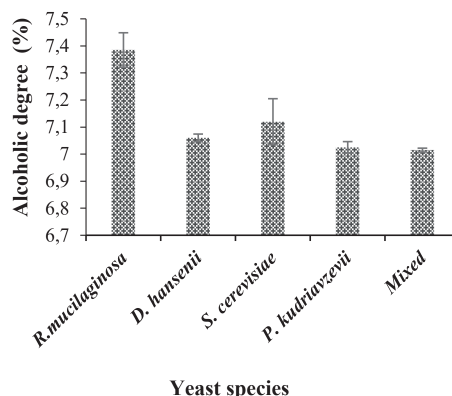

Figure 5. Alcoholic degree of distillates of yeasts isolates

Furthermore, the flasks containing the bioethanol produced are shown in Figure 6.

Figure 6. Flasks containing the bioethanol

Statistical analysis showed a significant difference between *Rhodotorula mucilaginosa* and the others strains. In our investigation, the ability of the yeast species to produce ethanol was used to assess ethanol resistance. Thus, the alcohol content of 7% v/v determined in our study was similar to that found by Yan et al. (2015), which was 7.34% (v/v), but far below the values found by Singh et al. (2013), which was 15.3% (v/v), by Ishola et al. (2015) with 37.1% (v/v), and by Moon et al. (2012) with 86.1% (v/v). This difference in alcohol content could depend on several factors, namely the ecological niche from which the yeast strain was isolated, the nature of the yeast (*Saccharomyces* and non-*Saccharomyces*), the fermentation conditions (temperature, stirring

speed, fermentation time, pH of the fermentation medium), and the raw materials (sorghum, maize, wheat, cassava, etc.) (Azhar et al., 2017). The strains used in this study were *Saccharomyces* species (*Saccharomyces cerevisiae*) and non-*Saccharomyces* species (*Debaryomyces hansenii, Rhodotorula mucilaginosa*, and *Pichia kudriavzevii*), with the fermentation conditions as follows: temperature: 25°C, stirring speed: 120 rpm, duration: 120 h and the raw material was sweet sorghum wort. Furthermore, the non-*Saccharomyces* yeast species showed the same capacity to produce ethanol as the *Saccharomyces* species and even more. Thus, the highest alcohol content was obtained with the non-*Saccharomyces* species, *Rhodotorula mucilaginosa*. For example, according to Mussato et al. (2012), the strains *Pichia stipitis* (NRRL-Y-7124) and *Kluyveromyces fagilis* (Kf1) were reported to be good ethanol producers from different types of sugars on par with the *Saccharomyces cerevisiae* species (RL-11).

Known to be an inhibitor of yeast growth and at the same time, a limiting factor for alcoholic fermentation, the mechanism of resistance to ethanol by yeast is the modification of their lipid composition to counteract these effects and avoid the permeabilization of their membranes (Stanley et al., 2010). According to You et al. (2003), adaptation to polar solvents such as ethanol results in an increase in unsaturated fatty acids and unsaturation levels. It is thought that the rise in unsaturated fatty acid content, especially C18:1 oleic acid, is a response to an adaptation to high ethanol concentrations. This allows for the stability of membrane integrity and the maintenance of metabolic stability and balance. This was also the same finding made by Coulibaly et al. (2018) when studying the resistance of yeast species to ethanol. Furthermore, other fat compounds such as ergosterol and phospholipids have also been cited as means of response to ethanolic stress (Swan et al., 1998; Inoue et al., 2000). Also, face to unable of yeast strains to produce ethanol at high concentrations, some yeast strains have been genetically modified (Alper et al., 2006). Location-specific mutagenesis has been used to create yeast capable of producing high levels of ethanol (Alper et al., 2006). The process involved changing specific words of the DNA code in a particular gene. However, the use of genetically modified microorganisms remains a controversial subject. Thus, certain non-*Saccharomyces* yeast strains were able to produce ethanol comparatively to *Saccharomyces cerevisiae* specie. *Debaryomyces hansenii* (Calahorra et al., 2009; Kurian et al., 2014); *Rhodotorula mucilaginosa* (Bura et al., 2012), and *Pichia kudriavzevii* (Ndubuisi et al., 2018; Akita et al., 2021; Pongcharoen, 2022) were used in bioethanol production.

CONCLUSIONS

Study of the ability of *Saccharomyces* yeast strain (*Saccharomyces cerevisiae*), non-*Saccharomyces* (*Debaryomyces hansenii, Rhodotorula mucilaginosa*, and *Pichia kudriavzevii*), and mixed culture (*Saccharomyces* and non-*Saccharomyces*) isolated form cocoa mucilage fermented juice, to produce bioethanol was investigated. All strains studied showed similar fermentation performances, but a difference was observed in alcohol content. Bioethanol content from different distillates ranged between 7.01±0.007 to 7.38±0.063% (v/v). The highest alcohol content was obtained with *Rhodotorula mucilaginosa* with 7.38±0.063% (v/v). The statistical analysis showed a significant difference (P<0.05) between *Rhodotorula mucilaginosa* and other strains and mixed culture. *Rhodotorula mucilaginosa* specie appeared as a suitable candidate to produce bioethanol. In further investigations, other raw materials from plants could be tested as substrates of fermentation.

REFERENCES

Akita, H., Goshima, T., Suzuki, T., Itoiri, Y., Kimura, Z., & Matsushika, A. (2021). Application of *Pichia kudriavzevii* NBRC1279 and NBRC1664 to Simultaneous Saccharification and Fermentation for Bioethanol Production *Fermentation*, *7, 83.*

Alexandre, H. (2014). Sélection de levures oenologiques Non-*Saccharomyces*. Institut Universitaire de la Vigne et du Vin Jules Guyot et Université de Bourgogne, Colloque flore microbienne d'intérêt dans les procédés alimentaires et la santé. Dijon, France.

Alper, H., Moxley, J., Nevoigt, E., Fink, G.R., & Stephanopoulos, G. (2006). Engineering yeast transcription machinery for improved ethanol tolerance and production. *Science*, *314*(5805), 1565-1568.

Antunovics, Z., Irinyi, L., & Sipiczki, M. (2005). Combined application of methods to taxonomic identification of *Saccharomyces* strains in fermenting botrytized grape must. *Journal of Applied Microbiology*, *98,* 971-979.

Azhar, M.S.H., Abdulla, R., Jambo, S.A., Marbawi, H., Gansau, J.A., Mohd, F.A.A., & Rodrigues, K.F. (2017). Yeasts in sustainable bioethanol production: A review. *Biochemistry and Biophysics Reports, 6(10)* 52-61.

Beavan, M.J., Charpentier, C., &Rose, A.H. (1982). Production and tolerance of ethanol in relation to phospholipid fatty-acyl composition in *Saccharomyces cerevisiae* NCYC 431. *Journal of General Microbiology, 128,* 1447-1455.

Birch, R.M., & Walker, G.M. (2000). Influence of magnesium ions on heat shock and ethanol stress responses of *Saccharomyces cerevisiae*. *Enzyme and Microbial Technology, 26,* 678–687.

Bura. R., Vajzovic. A., & Doty. S.L. (2012). Novel endophytic yeast *Rhodotorula mucilaginosa* strain PTD3 I: production of xylitol and ethanol. *Journal Industrial Microbiology and Biotechnology*, 39(7), 1003-1011.

Calahorra, M., Sánchez, N.S., & Peña, A. (2009). Activation of fermentation by salts in *Debaryomyces hansenii*. *FEMS Yeast Research, 9, (8),* 1293–1301.

Choi, G.W., Um, H.J., Kang, H.W., Kim, Y., Kim, M., & Kim, Y.H. (2010). Bioethanol production by a flocculent hybrid, CHFY0321 obtained by protoplast fusion between *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. *Biomass-Bioenergy*, *34,* 1232–1242.

Coulibaly, W.H., Cot, M., N'guessan, K.F., Coulibaly, I., Rigou, P., & Djè, K.M. (2018). Ethanol effect on yeast strains isolated from tchapalo, a traditional sorghum beer from Côte d'Ivoire. *International Food Research Journal*, *25(2),* 612-619.

Coulibaly, W.H., Bouatenin, K.M.J-P., Boli, Z.B.I.A., Camara, F., Sanogo, Y.M., Dogbo, M.A., Kouame H.K., Rigou, P., Clement Djameh, C., & Djè, K.M. (2021). Volatile compounds of traditional sorghum beer (tchapalo) produced in Côte d'Ivoire: comparison between wild yeasts and pure culture of *Saccharomyces cerevisiae*. *World Journal of Microbiology and Biotechnol*ogy, *37,* 75.

Demirbas, A. (2010). Use of algae as biofuel sources, *Energy Conversion and Management*, *51,* 2738–2749.

Dombek, K.M., & Ingram, L.O. (1986). Magnesium limitation and its role in apparent toxicity of ethanol during yeast fermentation. *Applied of Environmental Microbiology*, *52,* 975–981.

Gbohaida, V., Mossi, I., Adjou, E.S., Dossa, C.P.A., Wotto, D.V., Avlessi, F. & Sohounhloué, D.C.K. (2016). Évaluation du pouvoir fermentaire de *Saccharomyces cerevisiae* et de *Saccharomyces carlsbergensis* dans la production de bioéthanol à partir du jus de la pomme cajou. *Journal of Applied Bioscience*, *101 (1),* 9643–9652.

Groposilă-Constantinescu, D., Vişan, L., Mărgărit, G., Toma, R., Barba, C., & Hangan D. (2019). Higher utilization of waste from the food industry through biotechnological methods. *Scientific Bulletin. Series F. Biotechnologies, XXIII,* 143-146.

Hadiyanto, H., Ariyanti, D., Aini, A.P., & Pinundi, D.S. (2013). Batch and fed-batch fermentation System on ethanol production from whey using *Kluyveromyces marxianus*. *International Journal of Renewable Energy Development*, *2,* 127–131.

Hoekman, S.K. (2009). Biofuels in the US-challenges and opportunities, *Renew. Energy*, *34,* 14–22.

Inoue, T., Iefuji, H., Fujii, T., Soga, H., & Satoh, K. (2000). Cloning and characterization of a gene complementing the mutation of an ethanol-sensitive mutant of sake yeast. *Bioscience, Biotechnology and Biochemistry*, *64,* 229–236.

Ishola, M.M., Brandberg, T., & Taherzadeh, M.J. (2015). Simultaneous glucose and xylose utilization for improved ethanol production from lignocellulosic biomass through SSFF with encapsulated yeast. *Biomass Bioenergy*, *77,* 192–199.

Jiménez, J., & Van Uden, N. (1985). Use of extracellular acidification for the rapid testing of ethanol tolerance in yeasts. *Biotechnology and Bioengineering*, *27,* 1596–1598.

Kim, J.H., Ryu, J., Huh, I.Y., Hong, S.K., Kang, H.A., & Chang, Y.K. (2014). Ethanol production from galactose by a newly isolated *Saccharomyces cerevisiae* KL17. *Bioprocess and Biosystem Engineering*, *37 (9),* 1871-1878.

Kiran, B., Kumar, R., & Deshmukh, D. (2014). Perspectives of microalgal biofuels as a renewable source of energy. *Energy Conversion and Management*, *88,* 1228–1244.

Kurian, J.K., Minu, A.K., Banerji, A., & Kishore, V.V.N. (2014). Bioconversion of hemicellulose hydrolysate of sweet sorghum bagasse to ethanol by using *Pichia stipitis* NCIM 3497 and *Debaryomyces hansenii* sp. *Bioresources*, *5, (4),* 2404–2416.

Lai, Q.P. (2010). Utilisation de levures non-*Saccharomyces* en oenologie: études des interactions entre *Torulaspora delbrueckii* et *Saccharomyces cerevisiae* en cultures mixtes. PhD Thesis, Institut National Polytechnique de Toulouse, Toulouse.

Mardawati, E., Febrianti, E.A., Fitriana, H.N., Yuliana, T., Putriana, N.A., Suhartini, S., & Kasbawati. (2022). An integrated process for the xylitol and ethanol production from oil palm empty fruit bunch (OPEFB) using *Debaryomyces hansenii* and *Saccharomyces cerevisiae*. *Microorganisms*, *10,* 2036.

Moon, S.K., Kim, S.W., & Choi, G.W. (2012). Simultaneous saccharification and continuous fermentation of sludge-containing mash for bioethanol production by *Saccharomyces cerevisiae* CHFY0321*, Journal of Biotechnology*, *157,* 584–589.

Mossi, I., Adjou, E.S., Dossa, C.P.A., Nonviho, G., Adda, M.M.C., Yehouenou, B.B., & Sohounhloué, D.C.K. (2018). Production of bioethanol from stems of *Sorghum saccharatum* monitoring kinetic

parameters of fermentation. *Journal of Chemical Engineering and Materials Science*, *9(2),* 9-16.

Mussato, S.I., Machado, E.M.S., Carneiro, L.M., & Teixeira, J.A. (2012). Sugar metabolism and ethanol production by diferent yeast strains from cofee industry wastes hydrolysates. *Applied Energy, 92,* 763–768.

Ndubuisi, I.A., Nweze, J.E., Onoyima, N.J., Yoshinori, M., & Ogbonna, J.C. (2018). Ethanol production from cassava pulp by a newly isolated thermotolerant *Pichia kudriavzevii* LC375240. *Energy and Power Engineering*, *10,* 457-474.

Nigam, P.S., & Singh, A (2011). Production of liquid biofuels from renewable resources, *Progress Energy Combustion Science*, *37,* 52–68.

Novidzro, K.M., Agbodan, K.A., & Koumaglo, K.H. (2013). Study of the performance of four strains of *Saccharomyces cerevisiae* during the production of ethanol from enriched sucrose musts. *Journal de la Société Ouest-Africaine de Chimie*, *35;* 1-7.

Pongcharoen, P. (2022). The ability of *Pichia kudriavzevii* to tolerate multiple stresses makes it promising for developing improved bioethanol production processes. *Letters of Applied Microbiology 75 (1),* 36-44.

Safitri, R., Miranti, M., Astuti, I.D., Kartiawati, L., & Nurhayati, J. (2017). Efficiency of powder inoculum and microorganism encapsulation on hydrolyzate sugar fermentation of newspaper cellulose for bioethanol production. *Scientific Bulletin. Series F. Biotechnologies, XXI,* 216-222.

Saleh, A.K., Abdel-Fattah, Y.R., Soliman, N.A., Ibrahim, M.M., El-Sayed, M.H., Abd El-Aziz, Z.K., & El-Zawawy, W.K. (2022). Box-Behnken design for the optimization of bioethanol production from rice straw and sugarcane bagasse by newly isolated *Pichia occidentalis* strain AS. 2. *Energy and Environment*, 33 (8), 1613-1635.

Scordia, D., Cosentino, S.L., Lee, J.-W., & Jefries, T.W. (2012). Bioconversion of giant reed (*Arundo donax* L.) hemicellulose hydrolysate to ethanol by *Schefferssomyces stipitis* CBS6054. *Biomass-Bioenergy*, *39,* 296–305.

Singh, A., Sharma, P., Saran, A.K., Namita, S.B., & Narsi, R. (2013). Comparative study on ethanol production from pretreated sugarcane bagasse using immobilized *Saccharomyces cerevisiae* on various matrices. *Renew. Energy*, *50 (C),* 488-493.

Stanley, D., Bandara, A., Fraser, S., Chambers, P.J., & Stanley, G.A. (2010). The ethanol stress response and ethanol tolerance of *Saccharomyces cerevisiae*. *Journal of Applied Microbiology*, *109,* 13–24.

Swan, T.M., & Watson, K. (1998). Stress tolerance in a yeast sterol auxotroph: role of ergosterol, heat shock proteins and trehalose. *FEMS Microbiology Letters*, *169,* 191–197.

Thomas D.S., & Rose, A.H. (1979). Inhibitory effect of ethanol on growth and solute accumulation by *Saccharomyces cerevisiae* as affected by plasma-membrane lipid composition. *Archieves of Microbiology*, *122, 49-55.*

Tofighi, A., Assadi, M.M., Asadirad, M.H.A., & Karizi, S.Z. (2014). Bio-ethanol production by a novel autochthonous thermo-tolerant yeast isolated from wastewater*, Journal Environment Health Science and Engineering*, *12,* 107.

Yan, J., Wei, Z., Wang, Q., He, M., Li, S., & Irbis, C. (2015). Bioethanol production from sodium hydroxide/hydrogen peroxide-pretreated *anu* strain. *Bioresources Technology*, *193,* 103-109.

You, K.M., Rosenfield, C.L., & Knipple, D.C. (2003). Ethanol tolerance in the Yeast *Saccharomyces cerevisiae* is dependent on cellular oleic acid content. *Applied Environment Microbiology*, *69,* 1499–1415.

Zhao, J., & Xia, L. (2010). Bioconversion of corn stover hydrolysate to ethanol by a recombinant yeast strain, *Fuel Process Technology*, *91,* 1807–1811.