

Full Length Research Paper

Technological properties and sugar tolerance performance of palm wine yeasts isolated from parts of Nsukka, Nigeria

Nsikak U. Stephen^{1*}, Obeta J. Ugwuanyi² and Ukponobong E. Antia¹

¹Department of Microbiology, Akwa Ibom State University, Ikot Akpaden, Akwa Ibom State, Nigeria.

²Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Received 26 February, 2018; Accepted 18 June, 2018

The ability of yeasts to survive and produce significant ethanol in high sugar medium and high ethanol environment is essential for the use of such yeasts in industrial and edible ethanol production. Four *Saccharomyces* spp. strains ($S_1^t, n_1^t, u_1^t, k_1^t$) isolated from palm wine and an industrial strain were studied in high glucose medium for ethanol production and ability to survive in high sugar medium. *Saccharomyces cerevisiae* S_1^t produced remarkable cell concentration relative to other isolates and the industrial strain, IR-2 in 16, 24 and 36% (w/v glucose) fermentation broth. *S. cerevisiae* S_1^t survived well with good biomass yields of 2.21 and 6.74 fold in 24 and 36% w/v glucose broth, respectively. Ethanol fermentation at glucose concentration of 40% (w/v) produced 42.45 g ethanol concentration (P), 0.387g L⁻¹h⁻¹ volumetric productivity (Qp) and a yield (Yps) of 0.329 gg⁻¹. The sugar tolerance property was observed in a fermentation broth with an initial pH of 5.8. Additionally, *S. cerevisiae* S_1^t strain was adaptive to 10% ethanol in 24% glucose solution. The yield obtained and properties exhibited by this isolate compares outstandingly with published data for a range of industrially important isolates; thus, this isolate could be used to produce bioethanol in industrially sustainable processes.

Key words: *Saccharomyces*, bioethanol, osmotic stress, ethanol tolerance, sugar tolerance, indigenous yeast.

INTRODUCTION

Ethyl alcohol (ethanol, bio-ethanol) is a primary metabolite of yeast produced by fermentation of sugar. Yeast is used for the fermentation of simple sugar containing

substrate and polysaccharide that can be depolymerized to fermentable sugars (Rajasekaran et al., 2008). Yeast is a small-cell fungus that ferments sugars and reproduced

*Corresponding author. E-mail: nsikakstephen@aksu.edu.ng. Tel: +234 706 453 9888.

by budding (Walker, 2009).

The ability of yeast to thrive (ferment) in high sugar medium is one among other attributes required to qualify it for use in industrial ethanol production (Ogbonna, 2013). To ethanol producers, fermentation of high sugar substrate offers economic advantage in the production cost and yield that will be beneficial to the energy balance (Sanchez and Cardona, 2008; Puligundla et al., 2011). As known, the theoretical ethanol yield is 0.51 g in every 1 g of glucose (Bai et al., 2008). Thus, the higher the sugar concentration, the higher the ethanol yields.

However, successful fermentation of high sugar substrate is dependent on the yeast ability to withstand increased osmotic stress and to tolerate high ethanol concentration (Nuanpeng et al., 2011). Yeast cells exceed their normal sugar tolerance limit at more than 24 g/100 mL dissolved solids and thus limit the implementation of fermentation at elevated sugar concentration (Puligundla et al., 2011). The search for this sugar tolerance property in yeast strain has taken a center stage in ethanol research. Screenings for this property (fermenting power) have been done on a large number of strains isolated from grapes, fermenting grape musts and wines (Vaughan-Martini and Martini, 1998). So far, sugar tolerance trait tested and greater than 30% (w/v) has been identified in very few studies with hardly a study from Nigeria (Taing and Fumio, 1997; Scree et al., 2000, Erasmus et al., 2003; Bechem et al., 2007; Laopaiboon et al., 2009; Elizabeth et al., 2014).

Furthermore, bio-ethanol is currently the dominant renewable biofuel used in the transport sector (Sanchez and Cardona, 2008). It has already been introduced on a large scale in various countries such as Brazil and the US, and increasingly in European countries, and is now predominantly produced from sucrose-containing material such as cane molasses and starchy material (mostly grains). Also, at present, all beverage ethanol is made by fermentation (Sanchez and Cardona, 2008). Industrial ethanol is mainly manufactured by fermentation, but some are produced from ethylene by the petrochemical industry (Rajasekaran et al., 2008).

The main challenges in ethanol production at elevated sugar concentration is an effective industrial yeast which is expected to meet the criteria of sugar and ethanol tolerance, ability to thrive in concentrated solution, high overall volumetric productivity and high final ethanol (Slade, 2009). To achieve this target, organism must be able to grow in the inhibitory environment of high concentrations of sugar and other compounds, including ethanol (U.S. DOE, 2006). To enhance ethanol production, screening of palm wine yeast for sugar tolerance attribute is necessary. The aim of the present study was to evaluate the sugar tolerance performance of yeast strain (*Saccharomyces* genus) found in palm wine collected from Nsukka area of Enugu State, Nigeria and to evaluate the suitability of these yeast strains in ethanol production at elevated glucose concentration.

MATERIALS AND METHODS

Collection of sample

The palm wine samples used in this research study was from oil palm (*Elaeis guineensis*), purchased from palm wine tappers (inflorescence and stem tapping) in Nsukka areas (Opi, Ogurute, Udeno and Obukpa communities) of Enugu State. The palm wine was dispensed from the tappers container into a sterile sample bottle and transported immediately in an ice pack to the laboratory for analysis.

Yeast strain, media and culture conditions

Glucose peptone yeast agar (GPY) comprising of 8 g of glucose, 1 g of peptone, 1 g of yeast extract and 1.5 g of agar in 100 mL of sterile distilled water was used as growth medium in the culture of yeast cells from palm wine (Yarrow, 1998; Kurtzman et al., 2011). A ten-fold serial dilution was performed for the palm wine. An aliquot of 0.1 mL of 10^{-4} , 10^{-5} and 10^{-6} dilutions of the palm wine samples were cultured using spread plate technique and incubated at $27 \pm 2^\circ\text{C}$.

Single colonies were selected from GPY plate and purified by successive sub-culturing on GPY agar plates using streaking technique. They were preserved on slants of GPY media in the refrigerator at 4°C till needed.

Standardization of inoculums

Yeast strains selected as potential starter culture were standardized to obtain a uniform cell concentration to be used in subsequent experiment according to the methods of Moonja et al. (2003) and Zheng et al. (2012).

Phenotypic characterization of alcohol producing yeast strains

Twenty eight (28) yeast isolate and a typed strain *S. cerevisiae*-IR-2 (Accession no: DF 396938.1) were screened for ability to produce alcohol according to the method of Brooks (2008). Four alcohol producing strains were obtained, and identified according to the method of Yarrow (1998), Qureshi et al. (2007) and Kurtzman et al.

(2011) as *Saccharomyces S_1^t*, *Saccharomyces N_1^t*,

Saccharomyces U_1^t and *Saccharomyces K_1^t*. These four isolates and the type strain IR-2 were further screened for glucose tolerance.

Glucose tolerance test

Fermentation was carried out using borosilicate glass test tube of 15 mL capacity containing 12 mL of GPY broth at glucose concentrations of 240 and 360 g/L. Cells were inoculated at an initial O.D_{620} of 0.18 from cell suspension in ringer solution. At 3 h interval, a glass test tube was withdrawn and analyzed. The following analyzes were performed at each time interval: biomass determination, reducing sugar concentration and pH.

Genotypic characterization of yeast strain

Based on growth performance, isolate S_1^t was considered suitable for ethanol production studies at more than 360 g/L (w/v) glucose.

At this stage, the identity of the isolate was confirmed molecularly by sequencing the rDNA internal transcribed spacer region (ITS) using the method of Fietto et al. (2004).

Adaptation of yeast cells for combined glucose and ethanol tolerance

S. cerevisiae S_1^f strain was selected and prepared to cope with harsh environmental condition by adapting it to ethanol tolerance in high glucose solution to obtain a starter culture and ensured its viability at ethanol production at 400 g glucose/L. This was achieved in a stepwise manner of culturing the isolate at 24% w/v glucose, and then transferred to 36% w/v glucose and finally 40% w/v glucose. The yeast cells were harvested by centrifuging the culture at 4000 rpm for 5 min and suspended in ringer solution (Moonjai et al., 2003).

The strain was further adapted to ethanol tolerance at three different concentrations of ethanol (5, 10 and 15% v/v) in 240 g/L glucose solution using modified method of John and Watmore (1999). Samples were taken to analyze their viability using pour plate technique.

Ethanol production at 40% (w/v) initial glucose concentration

S. cerevisiae S_1^f cells were grown in synthetic medium containing (per litre): 400 g of glucose, 3 g of yeast extract, 5 g of peptone, 2.6 g of $(\text{NH}_4)_2\text{SO}_4$, 2.72 g of KH_2PO_4 and 0.2 g of $\text{Zn}(\text{NO}_3)_2$. The pH was adjusted to 5.2 using citric buffer (0.04 M) containing (per litre): 1.5 g of citric acid and 6.0 g of sodium citrate (Moonjai et al., 2003). Cells were inoculated at an initial O.D₆₂₀ of 0.18 which correspond to 6.6×10^9 cells/mL. The fermentation was carried out at $27 \pm 2^\circ\text{C}$ under static condition in duplicates for 120 h. At 12 h interval, a glass test tube was withdrawn and analyzed for biomass, reducing sugar concentration and ethanol concentration.

Analytical methods

Measurement of cell concentration

The change in biomass was estimated via optical density reading of the sample using colorimeter at 620 nm (Digital colorimeter, Model 312E, El products, India) and compared with a standard graph of optical density of the yeast cell versus cell concentration.

Glucose concentration measurement

The cell free extract obtained by centrifugation of the fermentation broth at 4000 rpm for 5 min was analyzed for total residual sugars by dinitrosalicylic acid method (Miller, 1959).

Measurement of pH

The changes in pH were measured using a digital pH meter (Hanna Instrument- H198107, pHep pH Tester, Italy).

Measurement of ethanol concentration

The ethanol concentration was estimated by iodine/thiosulphate method (A.O.A.C., 1980). The number of moles of thiosulphate titre volume was used to estimate the concentration of ethanol. The EBAS stoichiometry calculator software downloaded from

www.titrations.info/iodometric-titrations was used in calculating thiosulphate solution concentration.

Mathematical estimation

Change in biomass ($Y_{x/s}$)

The change in biomass ($Y_{x/s}$) was calculated as the actual viable cells produced and expressed as grams per gram glucose utilized (g g^{-1} glucose). The actual viable cell obtained in cells/ml was converted to grams based on the thumbs rule that one gram dry weight of yeast equates approximately 4.87×10^{10} cells (Russell, 2003).

Ethanol yield (Y_{ps})

The ethanol yield (Y_{ps}) was calculated as the actual ethanol produced and expressed as g ethanol per g glucose utilized (g g^{-1}).

Volumetric productivity (Q_p) and yield efficiency (E_y)

The volumetric ethanol productivity (Q_p) and the percentage of conversion efficiency or yield efficiency (E_y) were calculated by the following equations (Laopaiboon et al., 2008):

$$Q_p = \frac{P}{t} \text{ and } E_y = Y_p \times \frac{100}{0.51}$$

Where, P is the actual ethanol concentration produced (g L^{-1}), t is the fermentation time (h) giving the highest ethanol concentration and 0.51 is the theoretical yield of ethanol on glucose.

Statistical analysis

One way analysis of variance (ANOVA) comparison was performed using stata version 12 statistical software package. SPSS version 20 statistical software was used for graphical illustrations.

RESULTS

Screening/characterization of yeast strains

In the screening of 28 yeast isolates and a type strain (*S. cerevisiae* IR-2) for fermentative ability, 21 isolates were observed to be capable of gas production, while 7 isolates produced no gas in Durham's tube (Table 1). Four alcohol productive strains, one from each sample location and the type strain IR-2, were selected. The five selected yeast isolates S_1^f , k_1^f , n_1^f , u_1^f and IR-2 were identified to belong to the genus *Saccharomyces*. However, *Saccharomyces* spp. n_1^f and u_1^f yielded low biomass at 16% (w/v, glucose) and were thus screened out.

Growth studies of yeast isolates during glucose tolerance

The *S. cerevisiae* S_1^f growth response pattern in 24 and

Table 1. Screening results of yeast strains.

Column 1	Column 2	Degree of gas production	
S/N	Isolate	24hours	48 hours
1.	n_1^t	+++++	++++
2.	n_2^t	+++	++++
3.	n_3^t	++	++++
4.	n_4^d	++++	++++
5.	n_5^d	++	++++
6.	n_6^d	-	-
7.	n_7^d	++++	++++
8.	n_8^d	+++	++++
9.	s_1^t	++++	++++
10.	s_2^t	++++	++++
11.	s_3^t	++++	++++
12.	s_4^t	++++	++++
13.	s_5^d	++++	++++
14.	s_6^d	-	-
15.	s_7^d	++++	++++
16.	u_1^t	++++	++++
17.	u_2^t	+++	+++
18.	u_3^t	++++	++++
19.	u_4^t	-	+++
20.	u_5^t	-	+
21.	u_6^d	++++	++++
22.	u_7^d	-	+++
23.	k_1^t	++++	++++
24.	k_2^t	-	+++
25.	k_3^t	++	++++
26.	k_4^t	-	-
27.	k_5^t	++++	++++
28.	k_6^d	++++	++++
29.	IR-2	++++	++++

+++++: Very high gas production; ++++: high gas production; +++: moderate gas production; - no gas production; n^t : top palm wine from Ogurute; n^d : down palm wine from Ogurute; s^t : top palm wine from Opi; s^d down palm wine from Opi; u^t : top palm wine from Udenue; u^d : down palm wine from Udenue; k^t : top palm wine from Obukpa; k^d : down palm wine from Obukpa.

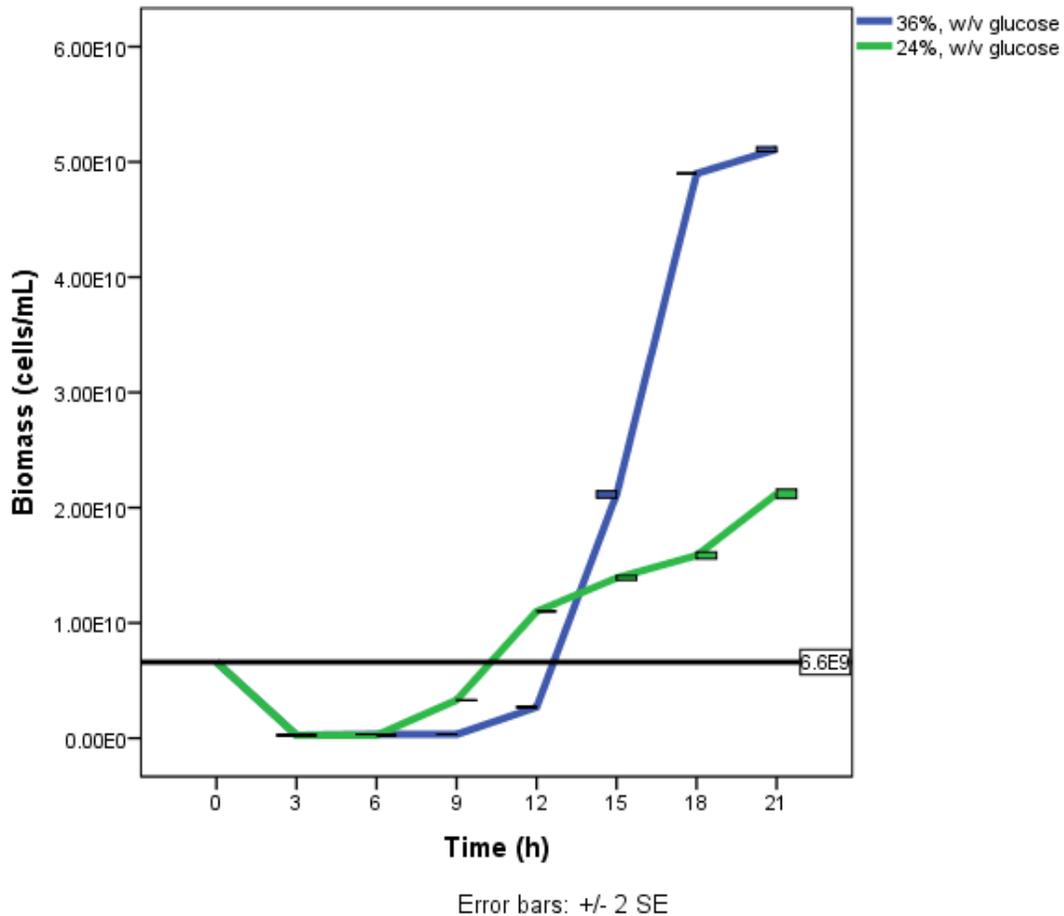


Figure 1. Response pattern of *S. cerevisiae* s_1^t strain in GPY medium.

36% w/v, glucose GPY medium during 21 h fermentation studies showed glucose tolerance qualities (Figure 1). At 24% (w/v glucose), the cell concentration increased 2.21-fold at the end of the fermentation (2.12×10^{10} cells/mL compared with 6.6×10^9 cells/mL initial concentration), while at 36%, the cell concentration increased 6.74-fold (5.11×10^{10} cells/mL when compared with 6.6×10^9 cells/mL initial concentration). However, at 36% glucose concentration, the lag phase of s_1^t strain last longer than at 24% glucose concentration. In contrast, k_1^t strain and IR-2 strain were unable to thrive at both concentrations of glucose tested (Figures 2 and 3). In the light of the growth response, k_1^t was not used in further studies.

***S. cerevisiae* s_1^t glucose consumption rate and change in biomass during 21 h fermentation**

The *S. cerevisiae* s_1^t strain utilized 42.91% glucose in

24% w/v, glucose with a corresponding biomass yield of 0.003 gg^{-1} (0.435 g when compared with 0.136 g initial biomass) (Figure 4). A residual sugar of 57.09% was *cerevisiae* s_1^t strain utilized 29.2% glucose with a corresponding biomass yield of 0.009 gg^{-1} (1.049 g when compared with 0.136 g initial). A residual sugar of 70.8% was observed.

pH profile of medium during 21 h fermentation

Figure 5 shows the pH profile of *S. cerevisiae* s_1^t strain during fermentation. The initial pH of the fermentation broth was 5.8. It was observed that in 24% (w/v) sugar, the pH dropped to 4.8 as compared to 5.2 at 36% (w/v) sugar. At both concentrations, the pH of the broth slightly increased within 6 h before decreasing until the end of fermentation period. Similarly, Figure 6 shows the pH profiles of IR-2 strain. In 24% (w/v, glucose concentration), a slight increase in the pH from initial Ph of 5.8 was observed at 6 h before decreasing to the initial pH of 5.8 at the end of the fermentation study.

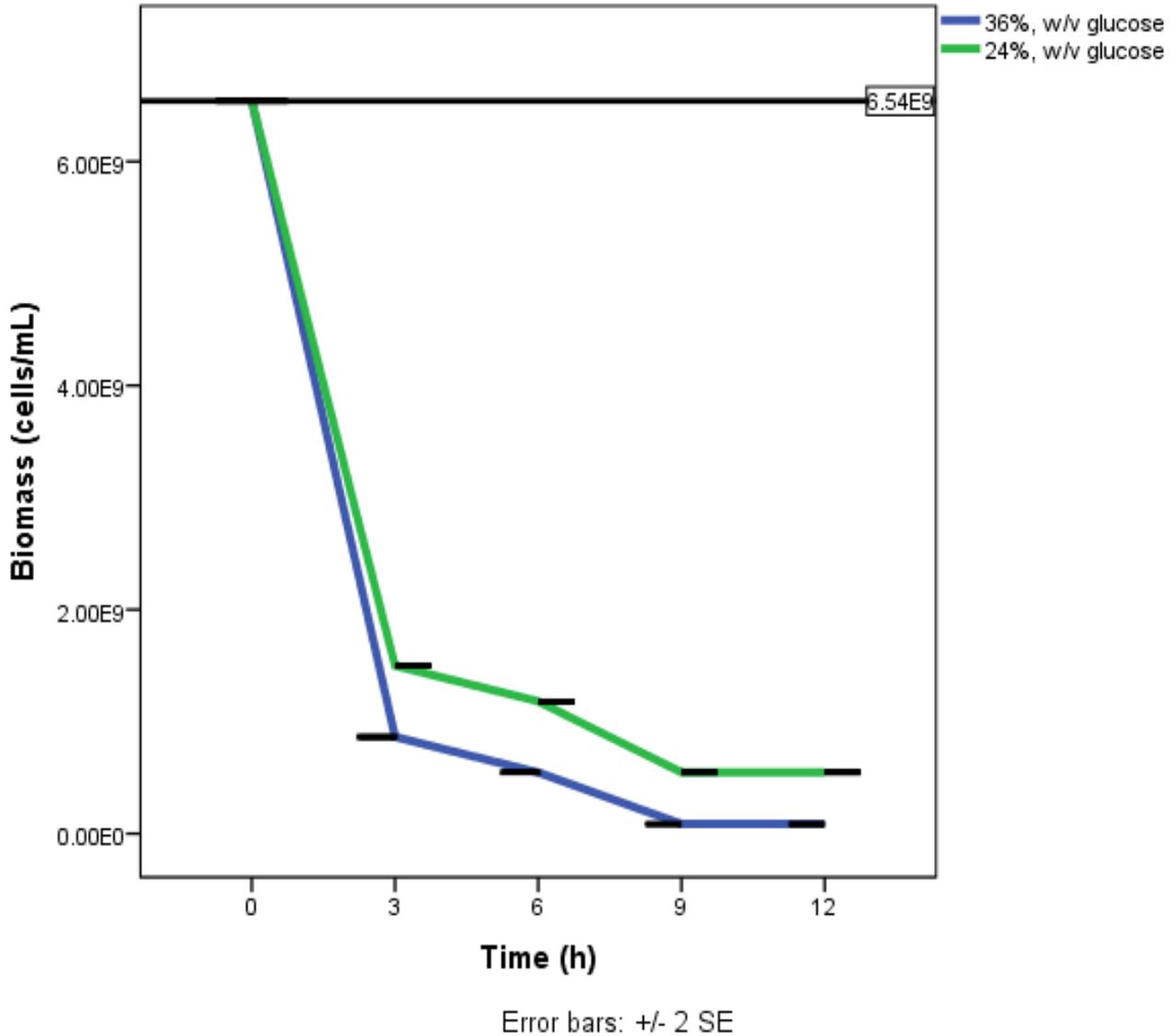


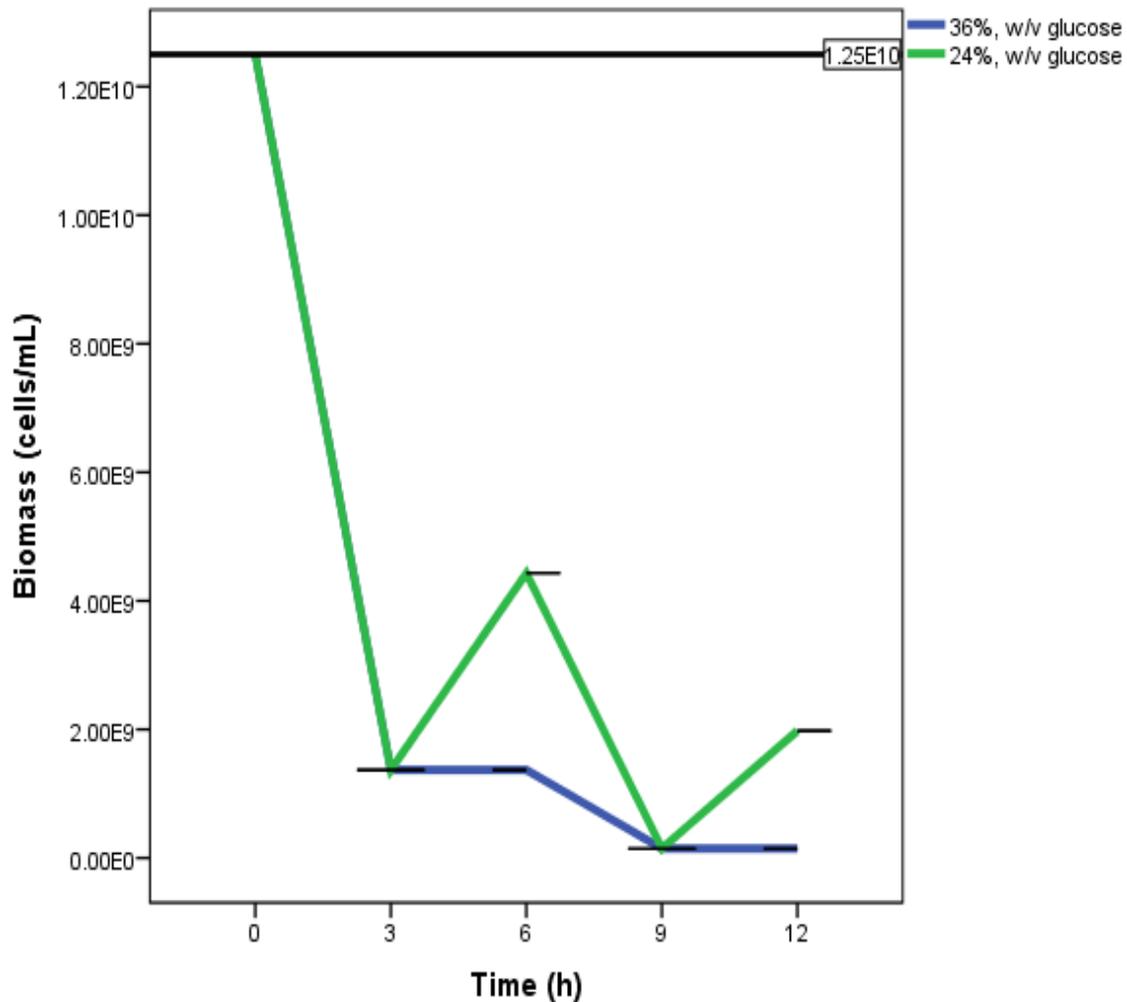
Figure 2. Response pattern of *Saccharomyces* sp. k_1^t strain in GPY medium.

Combined glucose and ethanol tolerance of *S. cerevisiae* S_1^t strain v/v.

The S_1^t strain was cultured at 24% w/v glucose supplemented with different concentrations of ethanol (5, 10 and 15% v/v). Figure 7 shows the growth response pattern at the different concentrations of ethanol. The total cell concentration produced at 0, 10 and 15% v/v were 6.15×10^9 , 1.11×10^{10} and 4.15×10^9 CFU/mL, respectively. These values were significantly lower than biomass at 5% v/v (2.30×10^{10} CFU/mL) ($P < 0.05$), but there was no significant difference in cell concentration at 0 (that is, when no ethanol was supplemented) and 15%

Ethanol production of *S. cerevisiae* S_1^t strain

Ethanol fermentation studies with *S. cerevisiae* S_1^t strain at glucose concentration of 40% (w/v) produced 46.45 g/L ethanol and productivity of 0.387 Lh^{-1} at 120 h fermentation period (Figure 8). The *S. cerevisiae* S_1^t strain utilized up to 32.25% glucose (Figure 9). Under this anaerobic condition, the cell concentration increased 10 fold (7.16×10^{10} cells/mL when compared with 6.6×10^9



Error bars: +/- 2 SE

Figure 3. Response pattern of *S. cerevisiae*-IR-2 strain in GPY medium.

cells/mL initial concentration) (Figure 10) and an ethanol yield of 0.329 gg^{-1} was observed.

Genotypic characterization of *S. cerevisiae* s_1^t strain

The yeast isolate was confirmed with partial 18S rDNA sequencing. The phylogenetic relationship of this isolates is shown in Figure 11. *S. cerevisiae* s_1^t is closely related to *S. cerevisiae* AD115 with 100% similarity.

DISCUSSION

Glucose tolerance

Out of the 28 palm wine yeast isolates assessed for the ability to ferment sugar to ethanol, 21 were positive.

These findings suggest that most yeast of palm wine were likely to have sugar fermentative tendency. Glucose at 24 and 36% w/v inhibited the growth rate of *S. cerevisiae* k_1^t and IR-2. Hence, it was needless to continue the fermentation experiment for 21 h run. In addition, there was no observed evidence of fermentation such as gas evolution; rather the cells died. According to Puligundla et al. (2011), some yeast fermentative ability and viability are severely compromised under high osmotic stress conditions. Similarly, Bonin and Skwira (2008) identified that high initial glucose-containing medium with sugar concentration of 200 to 300 g/L results in significant decrease of fermentation efficiency and yeast viability. However, high growth rate and fermentation rate was observed with the *S. cerevisiae* s_1^t strain. The strain had a biomass yield of 0.003 gg^{-1} in 24% (w/v) glucose concentrations which increased 2-fold in 36% (w/v)

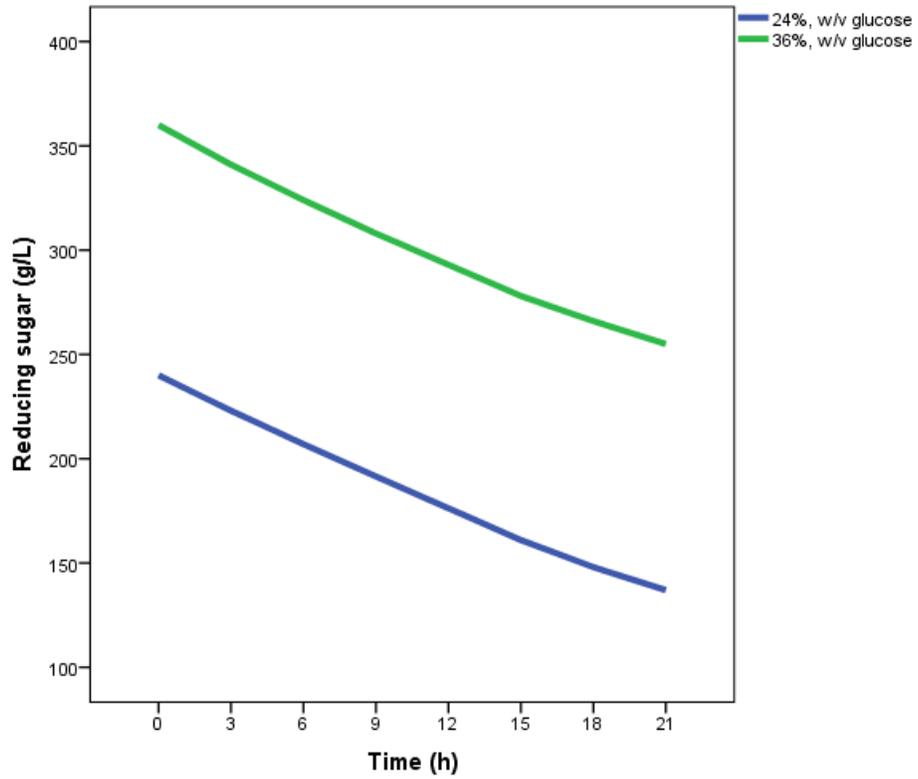


Figure 4. Glucose consumption pattern of *S. cerevisiae* strain in GPY medium.

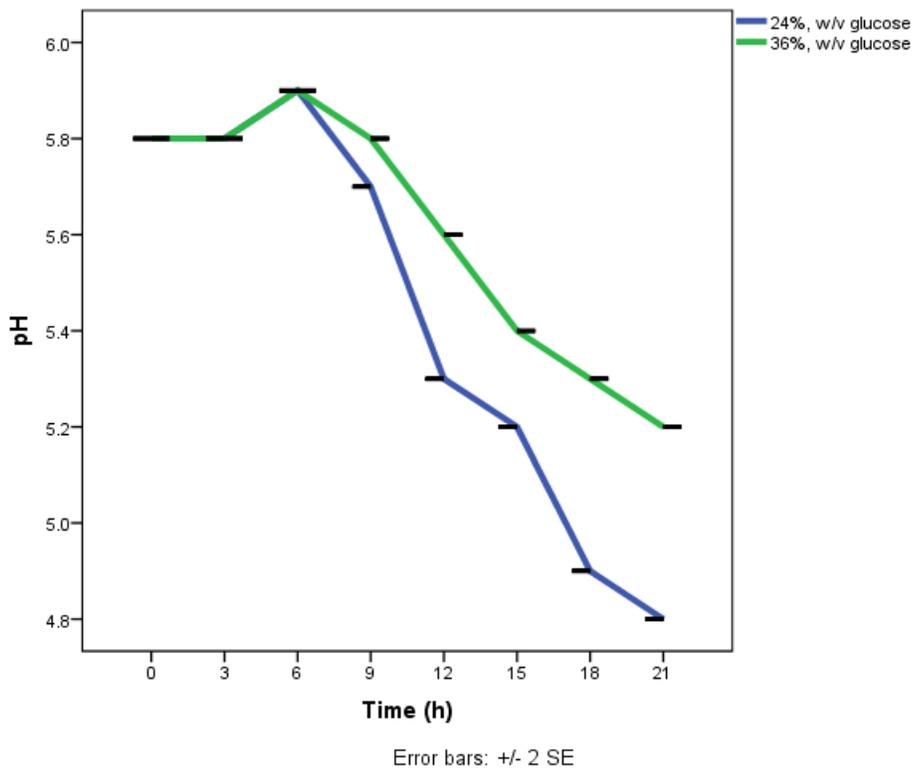


Figure 5. pH variation of *S. cerevisiae* strain in GPY medium.

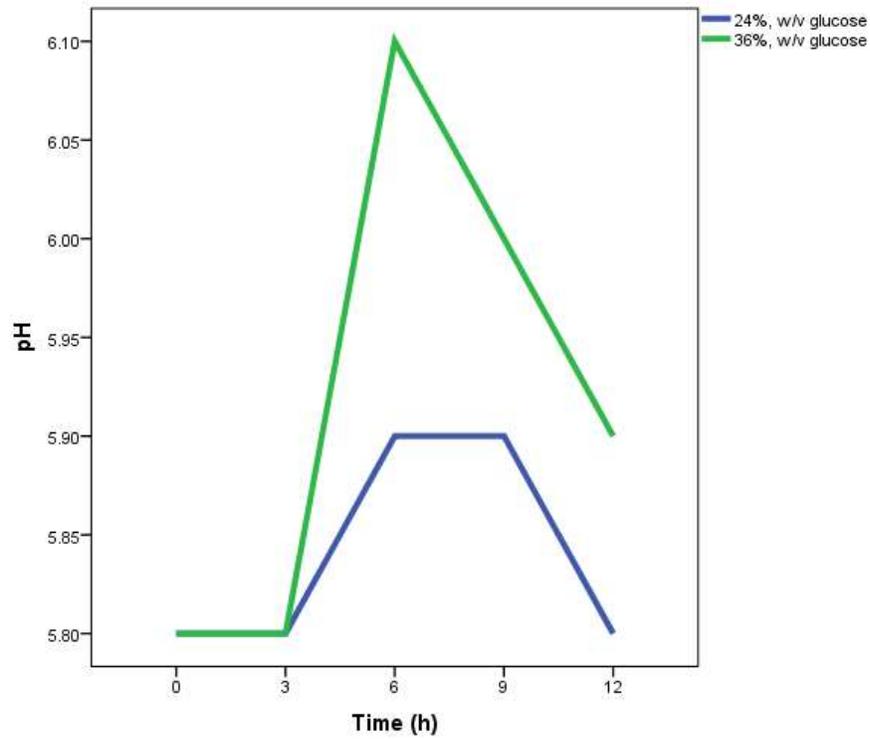


Figure 6. pH variation of *S. cerevisiae*-IR-2 strain in GPY medium.

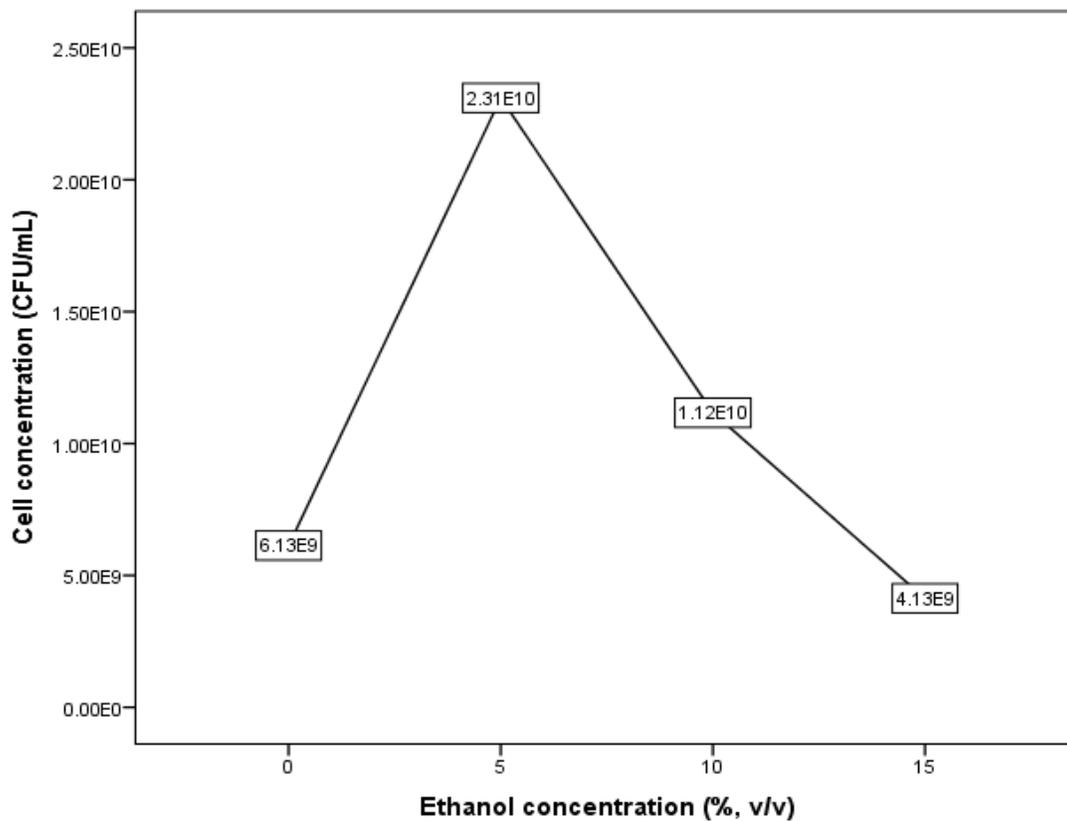


Figure 7. Growth response of *S. cerevisiae* strain during ethanol adaptation.

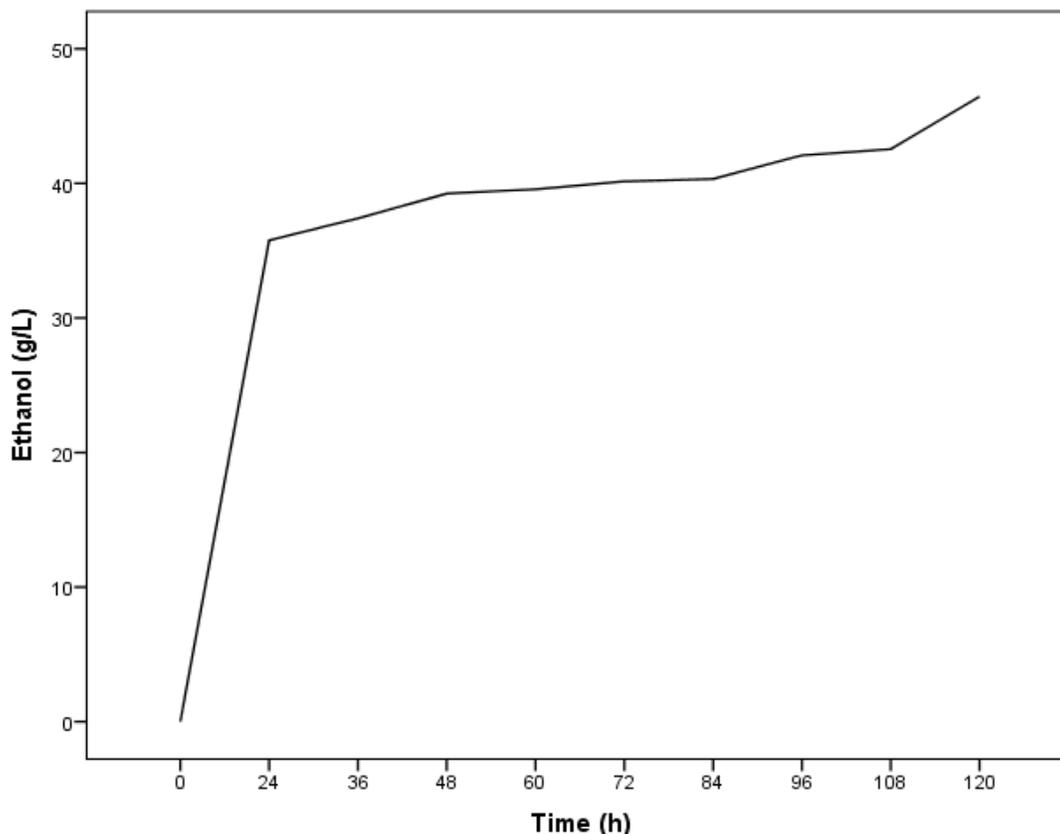


Figure 8. Ethanol productivity of *S. cerevisiae* strain in 40% (w/v) glucose ethanol production medium.

glucose concentration (0.003 as compared to 0.009 g g⁻¹). This physiological character is uncommon and has been reported in very few studies. Puria et al. (2009) reported on a yeast strain which was adaptive to 20 and 25% w/v glucose concentration. In Japan, 23 yeast strains were identified with the ability to grow on 50% w/v glucose and all but two strains grew on 60% w/v glucose medium (Taing and Fumio, 1997). In Cameroun, Bechem et al. (2007) found that 20% of the yeast strain from palm wine grew on 40% sucrose solution. Scree et al. (2000), reported on four *S. cerevisiae* from soil sample. They observed that all isolates were able to tolerate up to 350 g/L glucose. In a similar finding, Erasmus et al. (2003) observed yeast tolerance and growth in low water activity (40% w/v sugars) with a maximum specific growth rate of 0.023 h⁻¹. In Asia, Laopaiboon et al. (2009) observed yeast tolerance up to a concentration of 320 g/L. In Mexico, Elizabeth et al. (2014) assessed the osmotolerance properties in yeast strain in glucose media to be as high as 40% and their findings detected fermentative ability. To some yeast producers, sugar tolerance is a characteristic that varies the most between regions (Lallemand, 1996). Similarly, in the current research, finding supports these high sugar tolerance possibilities in yeast as the *S. cerevisiae* S₁^t strain held

up well under the 360 g/L glucose stress. However, at 36% glucose concentration, the lag phase of *S. cerevisiae* S₁^t strain lasted longer than at 24% glucose concentration. The difference may be due to the lowering of the water activity at higher glucose concentration. As known, sugar tolerance ability of yeast cells is critical in excess of glucose (>20% w/v). This is because as water concentration is lowered below the optimum level, the length of the lag phase increases and the growth rate decreases (Jay, 2005). Similarly, Osho (2005) identified that at increased sugar concentration of 20 to 25%, some strains of *Saccharomyces* species had prolonged lag phase of 12 h.

Combined glucose and ethanol tolerance

In this study, the result shows that the *S. cerevisiae* S₁^t strain was adaptive to 10% ethanol in 24% (w/v) glucose solution. From this observation, ethanol play a crucial substrate role in yeast propagation. The biomass yield improved significantly with supplementation at 5% ethanol, though yield tilted downwards at 10% ethanol concentration but it was statistically clear that the

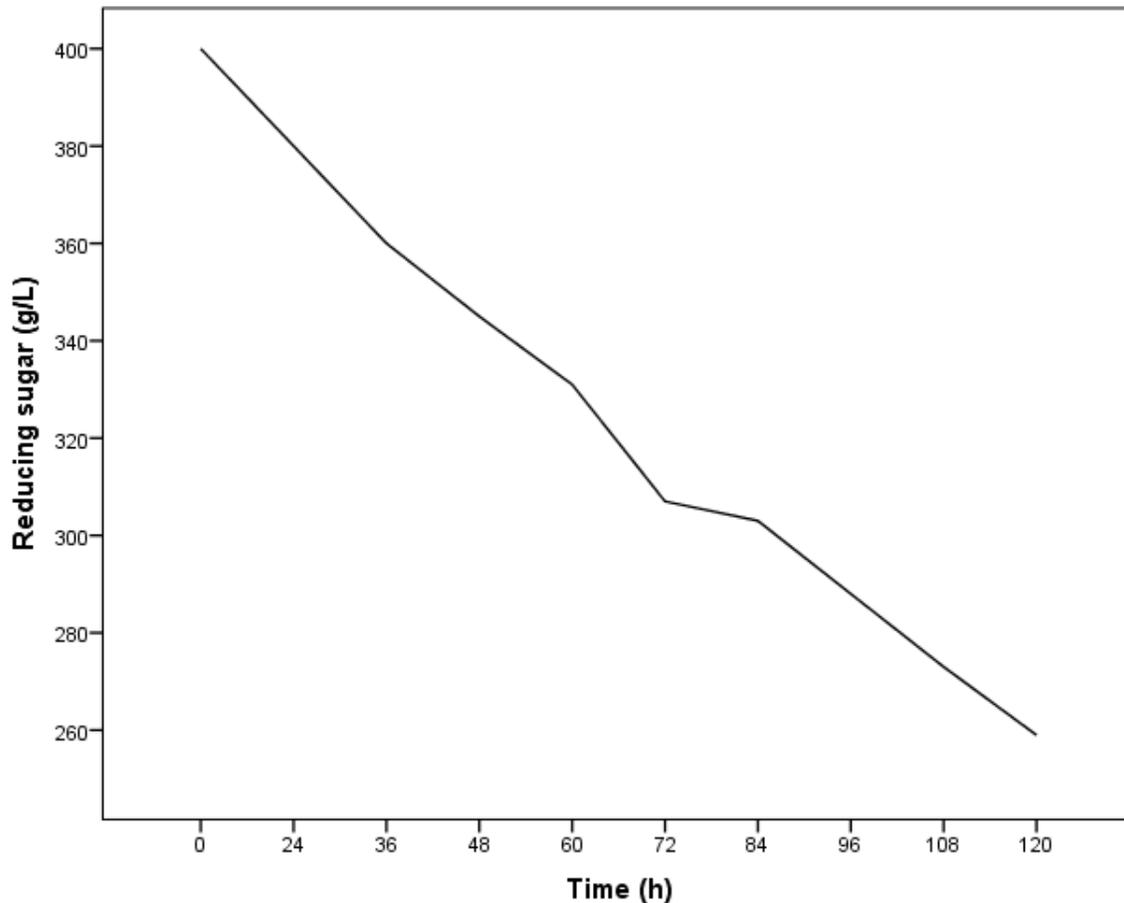


Figure 9. Glucose consumption pattern of *S. cerevisiae* strain in 40% (w/v) glucose ethanol production medium.

biomass yield was still greater than glucose solution without ethanol supplementation (0%). However, at 15% (v/v) ethanol viability decreased significantly. This result suggest the possibility *S. cerevisiae* S_1^t strain to thrive at high ethanol and glucose concentrations. Strobel and Lynn (2004) reported that an adapted strain of *Clostridium thermocellium* tolerated an ethanol concentration of 6 (wt/v) while the wild-type did not tolerate beyond 1.5 (wt/v) ethanol.

Ethanol production

Studies on high substrate fermentation have confirmed the fact that higher substrate concentration results in higher ethanol concentrations (Laopaiboon et al., 2008, 2009). A study by Laopaiboon et al. (2008) showed that at 240 g/L glucose concentration, the ethanol concentration produced with *S. cerevisiae* TISTR 5048 was 99.58 g/L and at 280, it was 99.42 g/L, at 320, it was 97.01 g/L while *S. cerevisiae* strain NP01 produced ethanol concentration of 101.95 g/L at 240, 104.680 g/L

at 280 and 104.68 g/L at 320. In the current study, the *S. cerevisiae* S_1^t yeast strain produced 46.45 g/L of ethanol at 400 g glucose/L with a productivity and ethanol yield of $0.387 \text{ g L}^{-1} \text{ h}^{-1}$ and 0.329 g g^{-1} , respectively, after five days cultivation. The result of ethanol fermentation in the current study with *S. cerevisiae* S_1^t strain affirms past findings on sugar tolerance and ethanol concentration of yeast cells at elevated sugar concentration.

Reducing sugar utilization by *S. cerevisiae* S_1^t

The glucose utilization rate appears slow as the sugar concentration increased. As known, ethanol inhibits cell growth, and also represses glucose transport (Salmon, 1989). In the current study, utilization of 42.91% was observed at 21 h fermentation in 24% (w/v) glucose concentration while utilization of 29.2% was observed at the same incubation time at 36% glucose initial concentration. In the course of 120 h ethanol fermentation, utilization of 32.25% was observed at 40%

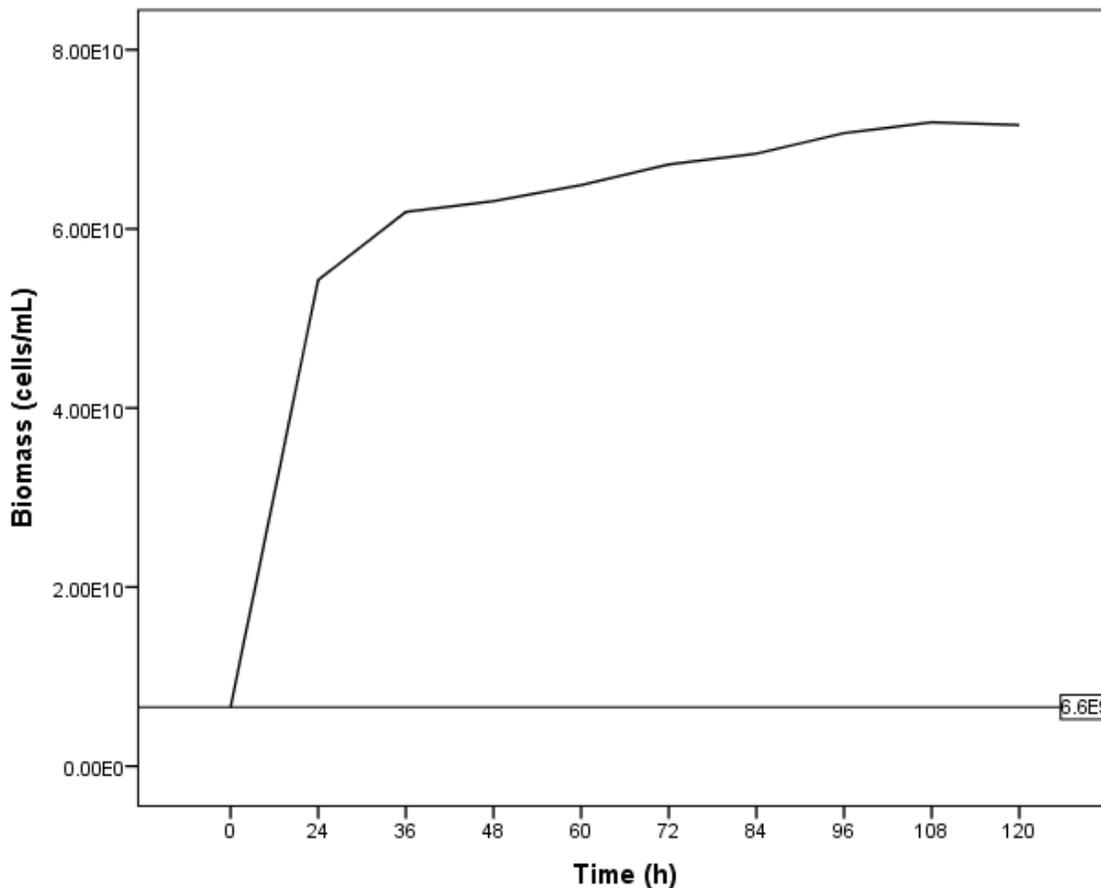


Figure 10. Growth extent of *S. cerevisiae* strain in 40% (w/v) glucose ethanol production medium.

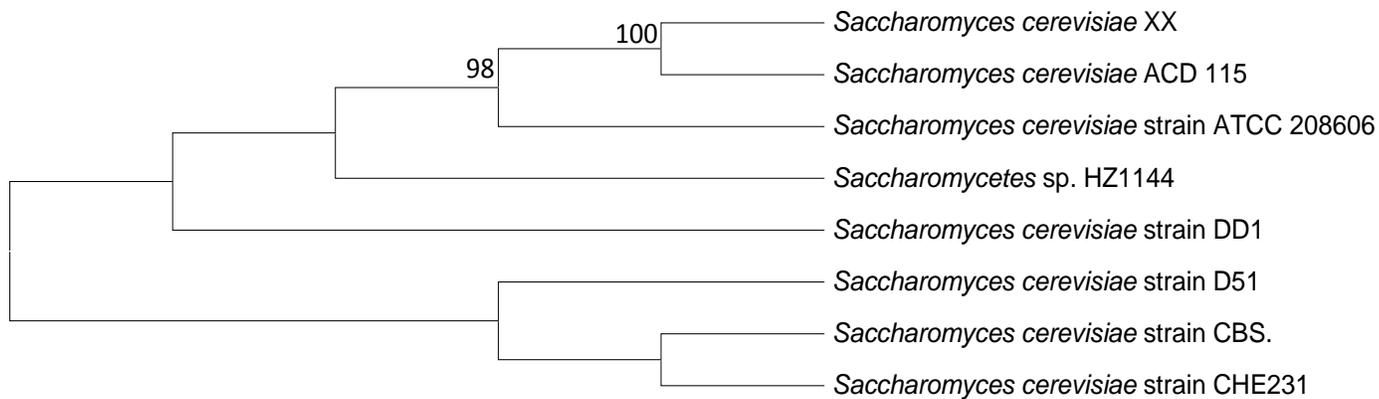


Figure 11. The phylogenetic tree of *S. cerevisiae* SCPW17. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. Evolutionary analyses were conducted in MEGA5.

(w/v) glucose initial concentration. This trend suggests a repressed glucose transport as the sugar concentration increased. In addition, the volumetric ethanol productivity with *S. cerevisiae* S_1^f strain ($0.387 \text{ gL}^{-1}\text{h}^{-1}$) was lower than

the expected for high substrate medium ($2\text{-}5 \text{ gL}^{-1}\text{h}^{-1}$) (USDOE, 2012). These suggest the possibility that the isolate may not be a good ethanol producer despite its sugar tolerant ability. As reported by Jay (2005) and Puligundla et al. (2011), *Saccharomyces rouxii* grew well

in high sugar media with a water activity of 0.6 but its ethanol production levels was relatively low. Nonetheless, in this current research, the *S. cerevisiae* S_1^f strain apparent ability to cope with the high initial glucose concentration was a good physiological trait. As also reported by Basso et al (2011), yeast strain that can ferment substrate with high productivity or at least cope with high substrate concentration even operating at normal ethanol titres is required in ethanol production.

Moreover, the *S. cerevisiae* S_1^f strain can come handy in high-sugar fermented food products for which sugar tolerant yeast could be employed during processing. For instance, a food product processed from fermentation of high sugar vegetables have been reported in Japan (Taing and Fumio, 1997). It could also be used in the fermentation of high fructose corn syrup (HFCS) and in alcoholic beverage production. In addition, one technology used in increasing brewing capacity is to ferment 18 g extract per 100 g liquid (18°P) to produce beers rather than the 12°P fermentation (Huuskonen et al., 2010; Puligundla et al., 2011) thus requiring a yeast strain such as *S. cerevisiae* S_1^f that tolerate high gravities.

Conclusions

The results obtained from the study have shown that isolate *S. cerevisiae* S_1^f strain is tolerant to high glucose-containing medium. The isolate was tolerant to concentration of glucose higher than 24% (w/v), which is a physiological character highly considered in yeast utilized as fermentation starters in ethanol industry. Based on these findings, the *S. cerevisiae* S_1^f strain proved to be a good choice for industrial ethanol production. Finally, the *S. cerevisiae* S_1^f strain sugar tolerance trait is of interest in some food industries which may be exploited. For maximum accumulation of ethanol by the *S. cerevisiae* S_1^f strain, further studies may be undertaken on metabolic engineering of the isolate.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors thank Prof. J. C. Ogbonna (University of Nigeria, Nsukka) for providing the *Saccharomyces*

cerevisiae-IR-2 strain.

REFERENCES

- Association Official Analytical Chemists (A.O.A.C.) (1980). *Official Methods Analysis*. Association of official analytical Chemists. Washington D.C. US.
- Bai FW, Anerson, WA, Moo-young M (2008). Ethanol fermentation technologies from sugar and starch feedstocks. *Biotechnology Advances* 26:89-105.
- Basso LC, Basso OT, Rocha SN (2011). *Ethanol Production in Brazil: The Industrial Process and Its Impact on Yeast Fermentation, Biofuel Production-Recent Developments and Prospects*, Dr. Marco Aurelio Dos Santos Bernardes (Ed.), ISBN: 978-953-307-478-8, InTech, Available from: <https://www.intechopen.com/books/biofuel-production-recent-developments-and-prospects/ethanol-production-in-brazil-the-industrial-process-and-its-impact-on-yeast-fermentation>
- Bechem EE, Omoloko C, Nwaga D, Titanji VP (2007). Characterization of palm wine yeasts using osmotic, ethanol tolerance and the isozyme polymorphism of alcohol dehydrogenase. *African Journal of Biotechnology* 6(14).
- Bonin S, Skwira J (2008). Effect of fermentation of high sugar fruit must on yeast cell. *Food Technology and Biotechnology* 46 (2):167-170.
- Elizabeth ML, Victor P, Santiago JT, Julio CC, Sergio RT (2014). Evaluation of ethanol production by osmotolerant yeast isolated from honey, cane syrups, molasses and other high sugar content substrate. Paper presented at the Mexiacan association for research in biotechnology. Mexico. Retrieved from <http://www.merb.edu>.
- Erasmus JD, George K, Merwe, VD, Hennie JJ, Vuuren Van (2003). Genome-wide expression analyses: Metabolic adaptation of *Saccharomyces cerevisiae* to high sugar stress. *FEMS Yeast Research* 3(4):375-399.
- Fietto JL, Araujo R S, Valadao FN, Fietto LG, Brandao RL, Neves, MJ, Gomes FC, Nicoli JR, Castro MI (2004). Molecular and physiological comparisons between *Saccharomyces cerevisiae* and *Saccharo myces boulardii*. *Canadian Journal of Microbiology* 50:615-621.
- Huuskonen A, Markkula T, Vidgren V, Lima L, Mulder L, Geurts W, Walsh M, Londesborough J (2010). Selection from industrial larger yeast strains of variants with improved fermentation in very-high gravity worth. *Applied and Environmental Microbiology* 76(50):1563-1573.
- Jay J (2005). *Modern Food Microbiology*. 4th Edition. Published by Satish Kumar Lairi. New Delhi-India.
- John S, Watmore B (1999). *Practical fermentation-a student's guide*. Published by the society for general microbiology. Available from <http://www.ncbe.reading.ac.uk>.
- Kurtzman CP, Fell JW, Boekhout T, Robert V (2011). *Methods for Isolation, Phenotypic Characterization and Maintenance of Yeast*. In: C.P. Kurtzman, J. W. Fell and TeunBoekhout (Ed). *The Yeasts, A Taxonomy Study*. 5TH Edition, Elsevier Publishers.
- Lallemand Inc., (1996). *Practical technology. Lallemand baking update*. 1 (3).
- Laopaiboon L, Nuanpeng S, Srinophakun P, Klanrit, P, Laopaiboon P (2008). Selection of *Saccharomyces cerevisiae* and Investigation of its Performance for Very High Gravity Ethanol Fermentation. *Biotechnology* 7(3):493-498.
- Laopaiboon L, Nuapeng S, Srinophakun, P, Klanrit, P, Laopaiboon, P (2009). Ethanol production from sweet sorghum juice using very high gravity technology: effects of carbon and nitrogen supplements. *Bioresource Technology* 100(18):4176-4162.
- Miller GL (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry* 31(3):426-428.
- Moonjai N, Verstrepen KJ, Delvaux, FR, Derdelincky, G, Verachtest H (2003). Unsaturated fatty acid supplementation of stationary-phase brewing yeast and its effects on growth and fermentation ability. Blackwell Science Publishers.
- Nuanpeng S, Laopaiboon L, Scrinophakun P, Klanrit P, Jaisil P, Laopaiboon B (2011). Ethanol production from Sweet Sorghum Juice under Very High Gravity Conditions: Batch, Repeated-batch and

- Scale up Fermentation. *Electronic Journal of Biotechnology* 14(1):1-12.
- Ogbonna JC (2013). *Industrial biotechnology, fundamental and bioprocess engineering for biotechnological production of useful metabolites*. 1st Edition. Isebu printing and publishing, Japan.
- Osho A (2005). Ethanol and sugar tolerance of wine yeasts isolated from fermenting cashew apple juice. *African Journal of Biotechnology* 4(7):660-662.
- Puligundla, PKS, Smvgrovicora, D, Obalam, VSR (2011). Very High Gravity (VTG) Ethanolic Brewing and Fermentation in a Research Update. *Journal of Industrial Microbiology and Biotechnology* 38:1133-1144.
- Puria R, Mannan MA, Chopra-Dewasthaly R, Ganesan K (2009). Critical role of *RPI1* in the stress tolerance of yeast during ethanolic fermentation. *FEMS Yeast Research* 9:1161-1171.
- Qureshi KS, Masud T, Sammi S (2007). Isolation and taxonomic characterization of yeast strains on the basis of maltose utilization capacity for bread making. *International Journal of Agriculture and Biology* 8:110-113.
- Rajasekaran R, Chadrasekaran R, Muthusilvan M (2008). Microbial biotechnology, rapid advances in an area of massive impact. *Advanced Biotechnology* 7(5):19-25.
- Russell I (2003). *Understanding yeast fundamentals, international center for brewing and distilling, school of life science, Heriot-watt University Edinburg, U.K.* In: Jacques, K.A., Lyons, T.P., Kelsall, D.R. (2003). *The alcohol textbook, a reference for the beverage, fuel and industrial alcohol industries*. 4th Edition. Nottingham University press
- Salmon JM (1989). Effect of sugar transport inactivation in *Saccharomyces cerevisiae* on sluggish and stuck enological fermentations. *Applied and Environmental Microbiology* 55:953-958.
- Sanchez JO, Cardona AC (2008). Trends in Biotechnological Production by Fuel Ethanol from different Feedstocks. *Bioresource Technology* 99:5270-5295.
- Scree NK, Smdhar M, Suresh K, Bharat IM, Rao LV (2000). High alcohol production by repeated batch fermentation using immobilized osmotolerant *Saccharomyces cerevisiae*. *Journal of Industrial Microbiology and Biotechnology* 24:222-226.
- Slade R (2009). Microbial fermentation-a budding opportunity for investment? *Cleantech magazine* (online). Retrieved from <http://www.cleantechinvestor.com>.
- Strobel HJ, Lynn B (2004). Proteomic analysis of ethanol sensitivity in *Clostridium thermocellum*. Paper presented in general meeting, American society for microbiology, New Orleans.
- U.S. Department of Energy (USDOE) (2006). Breaking the biological barriers to cellulosic ethanol. A joint research agenda. Retrieved from <http://www.doe.genomestolife.org/biofuels>
- Taig OK, Fumio H (1997). Identification of sugar-tolerant yeast isolated from high sugar fermented vegetable extracts. *Journal of General and Applied Microbiology* 43:39-47.
- Vaughan-Martini A, Martini A (1998). Determination of ethanol production. In: C.P. Kurtzman, J. W. Fell and TeunBoekhout (Ed). *The Yeasts, A Taxonomy Study*. 4th Edition, Elsevier Publishers.
- Yarrow D (1998). Methods for the Isolation, Maintenance and Identification of Yeast. In: C P Kurtzman & JW Fell (Ed). *The Yeasts, A Taxonomy Study*. 4th Edition, Elsevier Publishers.
- Zheng C, Sun X, Li L, Guan N (2012). Scaling up of ethanol production from sugar molasses using yeast immobilized with alginate-based MCM-41 Mesoporous Zeolite composite carrier. *Bioresource Technology* 115:208-214.