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# Bio-Prospecting Locally Relevant Industrial Yeast Strains from Fermented Carbohydrate-Rich Food Waste from Nigeria

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**Abstract**

Nigerian fermented food is characterized by high carbohydrate concentration. Widely spread yeast strains in the local fermented food waste amass traits useful to biofuel industry. This study aims to identify, characterize and assess the level of stress tolerance of local yeast strains residing in food waste produced from Nigerian "fufu," a fermented cassava product. Through the use of morphological, cellular, and amplification of TS1/ITS4 and D1/D2 rDNA sequencing, the yeast strains were identified. To test the yeast strains' resilience to stress, different concentrations of ethanol (15% - 20%v/v), sugar (5% - 25%w/v), and temperatures (30°C - 45°C) were applied. Out of the eight yeast strains, the isolate Y4, also known as *Kazachstania pseudohumilis* Y4 (Accession number: ON876535:1), was most promising since it could withstand ethanol at 15% and 20% (v/v). The strain showed tolerance to glucose at 20% (w/v) and temperatures (30°C-45°C). The carbohydrate-rich fermented food waste was a valuable source of stress-tolerant yeast, a vital resource for the bioethanol industry.

**Keywords:** Fermented food waste, *Kazachstania pseudohumilis* Y4, stress tolerant Yeast, ethanol, sugar, temperature

## 1.0 Introduction

Due to the excessive use of non-renewable fossil fuels brought on by the growing world population, greenhouse gases are released into the atmosphere, harming the ecosystem. To lessen the negative effects of an excessive reliance on fossil fuels, it is essential to source renewable energy as an alternative. One of the fuels with a renewable source is biofuel ethanol. The biofuel ethanol production have expanded over the years till date [1]. The fuel is clean, environmentally benign, and renewable both during manufacture and consumption. Bioethanol helps to reduce the amount of greenhouse gases released into the atmosphere as a result of the usage of fossil fuels worldwide.[2]. Bioethanol have been previously produced from agricultural residues, starch and sugar fermentation [3-5]. Recently, bioethanol production from kitchen waste has gained interest due to the enormous carbohydrate content which can be transformed to sugars for bioethanol production [6].

The effective microbial strain, adequate substrate, and bioprocess technology all contribute to the high yield of bioethanol, a byproduct of microbial fermentation. In addition to reducing viscosity, quick fermentation at relatively high temperatures also lowers the risk

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of contamination and the expense of cooling. [7, 8]. Since ethanol has been found to be an inhibitor of yeast cells, a high ethanol concentration may have an impact on the fluidity and toxicity of the membranes, as well as diminish cell viability and even cause cell death, [9], which in turn decreases the efficiency of conversion process. Therefore, thermo tolerance, ethanol tolerance, high sugar tolerance, and rapid rate of fermentation are crucial factors in choosing the right yeast for the manufacture of bioethanol. [10]. The rate of fermentation will remain constant if the sugar content is too high and exceeds the yeast cell's capacity to absorb it. [11]. However higher initial sugar concentration increases the fermentation rate and higher ethanol yield [12]. As a result, choosing a yeast strain that can handle high sugar levels will aid in increasing ethanol output and yield.

Many native yeast strains that can produce ethanol have been discovered from various sources, including dates [13], grape [14], animal feces [15, 16], sugarcane bagasse[17] [18]fermented goat milk [19], palm wine [20]. Although many bacteria are involved in food fermentation, yeast strains are the main players when it comes to the fermentation of starchy foods. The majority of the basic foods eaten in Nigeria are carbohydrate-rich grains and root tubers, some of which are consumed as fermented products. Various yeast strains have been reportedly isolated from Nigerian fermented foods such as fermented grains[21], cassava waste pulp [22], fermented cassava "Akpu" [23], millet sourdough [24]. To our knowledge, no research has been undertaken to evaluate yeasts isolated from waste from native fermented carbohydrate diets consumed in Nigeria for industrial value, such as bioethanol production.
The goal of this study was to isolate and identify the yeast strain from locally fermented carbohydrate food wastes in Nigeria using biochemical and molecular techniques, as well as to study the thermotolerance, sugar intake, and ethanol tolerances of yeast in order to improve strain resilience.

## 2.0 Materials and Methods

### *2.1 Preparation of food waste slurry*

A local food waste from" *fufu"* (An African "swallow" made from fermented cassava) was collected from food service center, University of Ilorin, Kwara State, Nigeria. Ten grams of the food waste sample was crushed with a laboratory mortar and pestle and then homogenized with 100 ml of sterile distilled water. The slurry was transferred into a clean 150 ml plastic bottle and covered. Fermentation was carried out at room temperature (30 ± 32°C) for ten days with frequent shaking at 2 days interval.

### *2.2 Isolation of yeast strains*

Yeast strains were isolated from the food waste slurry prepared in the previous step using spread plate method. Yeast Peptone Dextrose (YPD) Agar containing 1 % yeast extract, 2 % peptone, 2 % glucose, and 2 % agar was supplemented with 0.1 mg/ml streptomycin sulfate to inhibit bacterial growth. On YPD agar, aliquots of 0.1 ml from final dilutions of the diluted samples were spread. The plates were then incubated at 30°C for 48 h. The purified yeast strains were stored on YPD agar slant at 4 °C until further use. [25]

### *2.3 Morphological characterization and microscopy of yeast strains*

The yeast colonies isolated were differentiated based on their different cultural characteristics. The isolated yeast strains were characterized based on their colonial morphology (shape, margin, colour) [25]. As for cellular morphology (shape, budding), the yeast strain was cultivated in YPD broth for 24 h before viewing under the microscope at x 100 objective using classical methods [26].

*2.4 Stress exclusive tests*

*2.4.1 Ethanol tolerance test*

The ethanol tolerance test was carried out using a modified approach developed by Osho [27] and Karki, et al. [28]. Yeast isolates were cultured in YPD broth with 15% ethanol. The media was inoculated separately with 1 ml of cell suspension from each separated strain and the OD660nm 1.0 = 1.85 x $10^7$ cell/ml using a spectrophotometer (Vis S32A). The flasks were placed in a shaker incubator at 150 rpm and incubated for 72 hours at 30°C. The best four isolates were then exposed to a 20% ethanol concentration for an ethanol tolerance test.

*2.4.2 Thermo-tolerance test*

The standardized yeast isolates were inoculated onto the YPD agar and incubated at temperatures of 30, 37, and 45°C for 72 hours to demonstrate the ethanol-tolerant yeast's capacity to thrive at higher temperatures [29]. On the agar plate, the resultant growth intensity of the yeast strains was examined.

*2.4.3 Effect of time on glucose tolerance of the yeast isolates*

In the initial stage, YPD broth containing 5%, 10%, 15%, 20%, and 25% of previously filter-sterilized glucose was prepared for the glucose tolerance analysis. The medium was inoculated with 1 ml cell suspension of the yeast strain at $OD_{660nm}$ 1.0 = $1.85\times10^7$ cell/ml. Every 24 hours, the OD was monitored while all cultures were incubated at 30 °C for 144 hours. The incubation was halted as soon as each strain's optical density began to decline[30].

*2.5 Molecular Identification of yeast strain ($Y_4$)*

*2.5.1 Isolation of DNA*

Isolation of yeast DNA was done by adding 50 - 100 mg of yeast cells into a ZR BashingTM lysis tube containing 200 µl of water. Then 750 µl of lysis solution was added to the tube. The tube was secured in a bead fitted with 2 ml tube holder assembly and processed at maximum speed for 5 min. It was then centrifuged using a microcentrifuge (C100,Biomed) at 10,000 × g for 1 min. Thereafter, 400 µl of the supernatant was transferred to a Zymo-SpinTM IV Spin filter (orange top) in a collection tube and centrifuged at 7000 × g for 1 min and then filtered. A 1200 µl of fungal DNA binding buffer was added to the filtrate out of which 800µl of the mixture was transferred into a Zymo SpinTM IIC column in a collection tube and centrifuged at 10,000 × g for 1 min. Then 200 µl DNA Pre- Wash buffer was added to Zymo SpinTM IIC column in a new collection tube and centrifuged at 10,000 ×g for 1 min, following the addition of 500 µl fungal DNA wash buffer to the Zymo-SpinTM IIC column which was centrifuged at 10,000 rpm for 1 min. The Zymo-SpinTM IIC column was transferred to a clean 1.5 ml microcentrifuge tube and 100 µl DNA elution buffer was added directly to the column matrix, centrifuged at 10,000 rpm for 30 s to elute the DNA.

*2.5.2 Polymerase chain reaction*

The ITS1/ITS4 and D1/D2 rDNA Polymerase chain reaction (PCR) was performed for fungal detection. The primer pairs ITS1 and ITS4 (5′-TCCGTAGGTGAACCTGCGG-3′ and 5′- TCCTCCGCTTATTGATATGC-3′) and LR0R and LR7 (5′ - ACCCGCTGAACTTAAGC-3′ & 5′-TACTACCACCAAGATCT-3′) were used for PCR, respectively [31, 32]. In every PCR, a negative control (distilled water) and a positive control (fungal genomic DNA) were included.

2.5.3 *Library preparation and nanopore sequencing*

When the PCR bands were observed by gel electrophoresis, sequencing libraries were prepared using the PCR products. When the PCR band was too weak to draw a conclusion, 40 cycles of PCR were repeated, and the PCR products were reevaluated by gel

electrophoresis. Libraries were generated using the Rapid Barcoding Sequencing kit (SQK-RBK004) following the manufacturer's protocol (Oxford Nanopore Technologies, Oxford, UK). Then, nanopore sequencing was performed on GridION for 2 h.

*2.5.4 Analysis of amplicon sequencing*

After sequencing, the generated reads were analyzed with EPI2ME (Oxford Nanopore Technologies, Oxford, UK), a cloud-based data analysis platform. The WIMP workflow was used for the analysis of ITS1 or D1/D2 rDNA amplicons. The WIMP workflow of EPI2ME offers taxonomic classification of the reads based on NCBI RefSeq database (https://www.ncbi.nlm.nih.gov/refseq/) The phylogenetic and molecular evolutionary analyses were conducted with MEGA version 7 [33] using the neighbour-joining method [34, 35].

## 3.0 Results and discussion

*3.1 Isolation and characterization*

In this study yeast strains were isolated from a non -studied substrate such as local food waste from *"fufu"*. A total of 8 different yeasts strain ($Y_1$, $Y_2$, $Y_3$, $Y_4$, $Y_5$, $Y_6$, $Y_7$, and $Y_8$) were from "*fufu*" and presented different cultural and morphological characteristics. Yeast strains with the ability to ferment ethanol that have been isolated from various sources have been documented in previous research. The yeast strain were isolated from sugar factory bio waste, [17], soil [36], kitchen waste (vegetable and fruits and sugar) [10]. Over 80% of the colonies in the current study had smooth surfaces, with colonies ranging in size from small to big creamy colonies dominated the study. The isolates' morphological traits are shown in Table 1.

**Table 1: Morphological characterization of the yeast isolates**

| | Y1 | Y2 | Y3 | Y4 | Y5 | Y6 | Y7 | Y8 |
|---|---|---|---|---|---|---|---|---|
| **Color** | Cream | White | Cream | Cream | Cream | white | Cream | Cream |
| **Opacity** | Opaque | Opaque | Opaque | Opaque | Opaque | Opaque | Opaque | Opaque |
| **Surface** | Rough | Rough | Smooth | Smooth | Smooth | Smooth | Rough | Smooth |
| **Margin** | Cream | Undulate | Undulate | Undulate | Undulate | Entire | Entire | Undulate |
| **Elevation** | Flat | Flat | Flat | Raised | Raised | Flat | Flat | Flat |
| **Size** | Large | Large | Large | Small | Small | Large | Large | Large |
| **Form** | Irregular | Irregular | Irregular | Irregular | Irregular | Irregular | Irregular | Irregular |
| **Texture** | Butyrous | Butyrous | Butyrous | Butyrous | Butyrous | Butyrous | Butyrous | Butyrous |
| **Filament** | No Hypae | No Hypae | No Hypae | No Hypae | Pseudo Hypae | Pseudo Hypae | No Hypae | No Hypae |
| **Asexual reproduction** | Budding | Budding | Budding | Budding | Budding | Budding | Budding | Budding |
| **Cell shape** | Lemon | Lemon | Cylindrical | Oval | Cylindrical | Lemon | Oval | Roughly cylindrical |
| **Ascospores shape** | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| **Asexual reproduction** | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |

Typical of yeast strains, the asexual form of reproduction, termed budding was observed in the photomicrograph (Figure 1) with spherical, oval and roughly cylindrical shape. Yeast with similar characteristics with the ones reported in this study have been reported previously [14, 17].

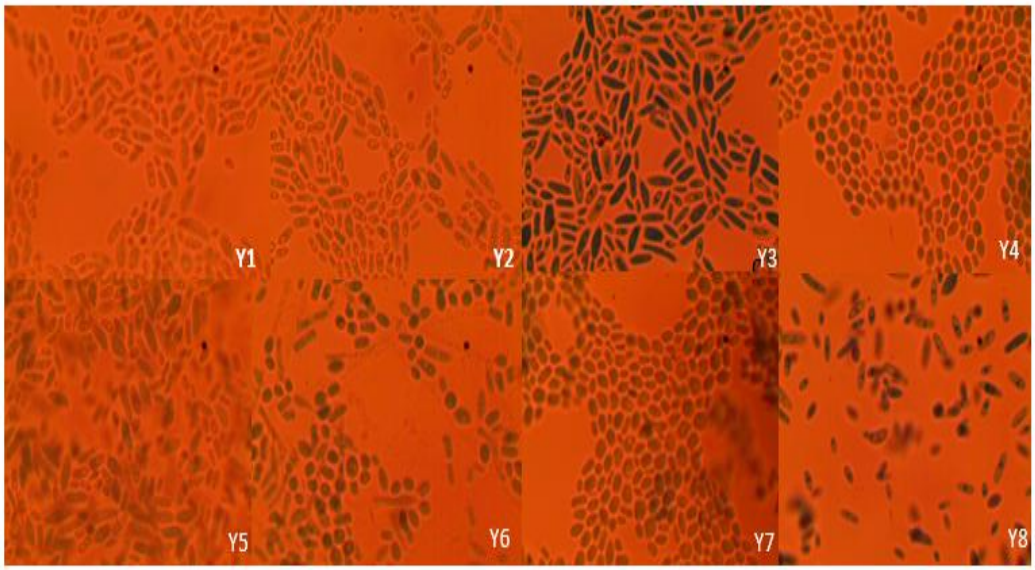


**Figure 1:** Photomicrograph (X100 objective) of isolated yeast strains (Y1 to Y8) after 24 h cultivation on YPD broth.

*3.2 Ethanol tolerance tests*

The ethanol tolerance profile of the eight yeast isolates subjected to ethanol concentration of 15 % is presented in Figure 2a. Most of the yeast strains displayed tolerance to 15 % ethanol concentration up to 72 h with isolates Y1, Y4, Y5, Y7 and Y8 showing higher tolerance. The findings are in line with those of [37] who claimed that *Sacharomyces cerevisiae* UVNR56 was tolerant to 15% ethanol concentration. High ethanol concentration generates heat shock- like proteins, disrupt metabolism, and denature and inactivate glycolytic enzymes [38], These effects reduce cell survival and growth, which in turn reduces productivity [39]. Additionally, yeast that is tolerant to high ethanol and has increased fermentation performance is best taken into account in industrial fermentation [9]. The yeast's ethanol tolerance in this study was slightly higher than those reported by other researchers.

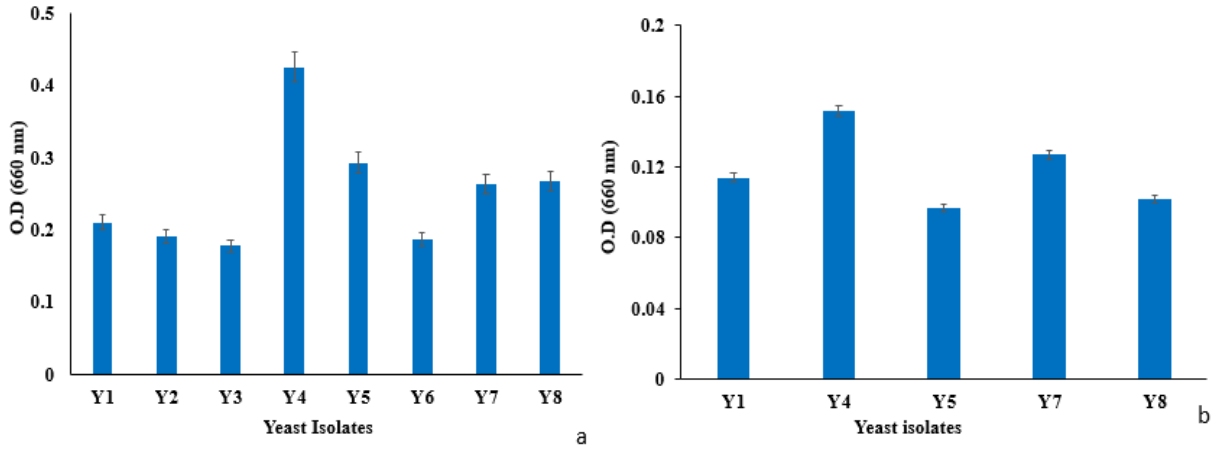


**Figure 2:** Ethanol tolerance profile of yeast strains at 15 % (v/v) (a), and 20 % (v/v) ethanol (b).Values are mean (n=3)±SD

The three yeast isolates (Y1, Y4, and Y7) could tolerate 20% of the ethanol concentration (Figure 2b), with Y4 showing the greatest tolerance. The outcome is comparable to that of the yeast strains MJTm3 and S1&S2 that were reported by [13, 17].Overall the lowest tolerance to ethanol was recorded by Y3. The ability of the yeast strains to grow in media with rather high ethanol concentrations (15% and 20%) indicates that they can survive osmotic stress. In contrast to earlier studies, the soil-isolated yeast strain Z087A0VS was able to withstand up to 11% ethanol [14]. Meanwhile, Htet, et al. [15], reported that the yeast strain only grew slowly at ethanol concentrations above 5%. Ellyastono and Harsojo [40], additionally observed tolerance to ethanol at concentrations as low as 5% in a mutant yeast. The diverse origins of the substrates, geographic locations, and genetic variances of the isolates may all contribute to the yeast strains' varying ability to tolerate ethanol.

*3.4 Temperature tolerance test*

Yeast strain activity and viability are influenced by growth temperature. The cultivation of yeast strains under the various temperatures is highlighted in Table 2. The findings indicate that all isolates have a strong growth response at 30 °C. The ideal temperature for *Saccharomyces cerevisiae* has been reported to be between 25 and 30 °C [41]. Karki, et al. [28], revealed that the yeast strain isolated from diverse fruit sources showed maximum growth at 25°C and 30°C. Depending on their isolation origin, certain yeast may thrive well at temperatures greater than the average. In this investigation, a moderate temperature tolerance was found to exist at 37 °C. The outcome of this investigation is comparable to that reported by Phong, et al. [36] who reported that all isolated yeast strains from soil were temperature resistant at 37 °C. High temperatures can reduce the risk of contamination and the expense of cooling, making the production of ethanol at high temperatures advantageous in industrial settings. Because of their ability to boost ethanol fermentation efficiency, yeast strains with thermotolerant capacity should be sought for. Yeast strain Y4 demonstrated high temperature tolerance at 37 °C and moderate temperature tolerance at 45 °C. According to [15] seven out of 22 yeast strains isolated from different source were temperature at 45 °C.

**Table 2:** Temperature tolerance test of yeast strains isolated from fermented "fufu" waste

| Yeast strain | Growth temperatures °C | | |
|---|---|---|---|
| | 30 | 37 | 45 |
| **Y1** | +++ | ++ | + |
| **Y4** | +++ | +++ | ++ |
| **Y5** | +++ | ++ | - |
| **Y7** | +++ | ++ | - |
| **Y8** | +++ | ++ | + |

+++ = Intensive response
++ = Moderate response
+ = Low response
- = No response

Temperature tolerance in yeast strains has been attributed to mutations brought on by repeated exposure to higher temperatures, which result in the gain of the high temperature growth related gene (Htg+).[42]. Nevertheless, Prado et al. [43], stated that yeast thermotolerance was characterized by a change in metabolic route during high-temperature fermentation, which further strengthens tolerance to high ethanol, carbohydrates, and inhibitor concentrations. It was observed that yeast strain Y1 and Y8 showed a low response to growth at 45°C. As the temperature rises to 40°C, the growth of the yeast strains Y2, Y3,

Y5, Y6, and Y7 is severely impeded. Higher temperature enzymes that catalyze the rate of biological reactions may be denatured, increasing activation energy and reducing the yeast strain's growth rate. An organism may have delayed metabolic activity, cell damage, and eventual death if the temperature is higher than it can tolerate.

*3.5 Glucose tolerance test*

Figure 3(a-h) shows how different glucose concentrations affect the growth of the isolates at different time. Different yeast strains displayed varying degrees of tolerance to glucose at various doses. In general, yeast growth increased steadily as fermentation time increased up until the maximum (between 96 h and 120 h), after which growth declined. As evidenced by yeast strains Y1, Y2, Y5, Y6, Y7, and Y8, the growth rate remains the lowest at a sugar concentration of 25%. At glucose concentrations of 20% and 25%, early growth declination was seen, as shown in Figure 3a. The Y 2 strain grew more effectively up to 120 hours at a lower glucose content of 5% ( Figure 3b). While optimal growth (OD 1.428) was obtained at 15% glucose concentration, growth started to decline at 20% glucose concentration after 96 hours. The log phased was kept at 5, 10, and 15%, though the maximum growth (O.D. 1.482).was seen at 15% glucose concentration. The highest rate of growth was still attained at a glucose concentration of 15%, however, the log phased was extended to a glucose concentration of 20%. Early growth declination at 25% glucose concentration was first noticed at 72 hours, however prolonged incubation increased the yeast growth until 120 hours (Figure 3d). At a 20% glucose concentration, the isolate Y4 achieved O.D. of 1.587 at 120 hours. The increase in growth rate at all concentrations that only started to diminish after 120 h (Figure 3d) distinguishes Y4's tolerance behavior from that of other isolates. After 96 hours, yeast Y5's growth was inhibited by glucose concentrations of 15%, 20%, and 25% ( Figure 3e). At low concentrations of 5 and 10%, the isolate exhibited tolerance to glucose. Only 96 hours of yeast Y6 growth could be sustained at glucose concentrations of 5%, 15%, and 25% (Figure 3f). However, a 10% glucose concentration resulted in the maximum yeast growth at O.D 1.525. With the exception of 20% glucose concentration, the yeast strain Y7 showed greater sensitivity to glucose at 5%, 10%, 15%, and 20% for 96 hours (Figure3g). At a glucose concentration of 20%, the best growth was observed, but a concentration of 25% indicated the least tolerance. Figure3h shows that the yeast Y 8 similarly had a limited tolerance for glucose concentrations of 25%. However, at 120 hours, 20% glucose concentration resulted in optimum development (OD 1.501). Nevertheless, at 15% glucose concentration, early growth declination was seen. For yeast fermentation, which produces energy for metabolism, glucose is a crucial carbon source. Due to glucose depletion, the yeast entered a stationary phase, which caused the observed decline in growth. The lower O.Ds found with yeast Y1, Y2, Y5, Y6, Y7, and Y8 at 25% glucose concentration indicated that the yeast strains' growth and persistence were lowered in the fermentation flask. High substrate concentration according to Bisson and Fraenkel [44], inhibits yeast growth and glycolytic enzyme inhibition. Due to osmotic pressure, high glucose concentrations restrict yeast growth [14]. Maslanka and Zadrag-Tecza [45], however, observed that some yeast strains have high glucose absorption, as evidenced by high glycolytic flux and a faster rate of biosynthesis. Furthermore, the differences in glucose tolerance of the yeast strains could also be attributed to the differences in their genetic makeup. Furthermore, the differences in glucose tolerance of the yeast strains could also be attributed to the differences in their genetic make-up. Furthermore, at the early growth phase up to the stationary phase, increasing yest cells yield and utilization was observed. Despite this increase, for all samples, after 120 h of fermentation, cell viability declined. Similar observation has been reported for yeast cell viability decline for all samples, after 192 h of fermentation. Consequently, the synergistic action of other byproducts at 120 h to 144h, has also been reported to impact negatively on the cell viability, with a larger decrease in viable cell count for fermented

samples [46]. Other observations, have reported a constant decrease of cell viability during the fermentation process after 192h, and the differences observed were attributed to the different cell NaCl pre-culturing time, and the different volume of fermented medium [47].The longest exponential time for all yeast strains between 48 h to 120 h showed higher glucose utilization. However, in other reported observations, the latency phase and exponential phase showed moderate glucose concentrations utilization at 14 h to 16 h [48]

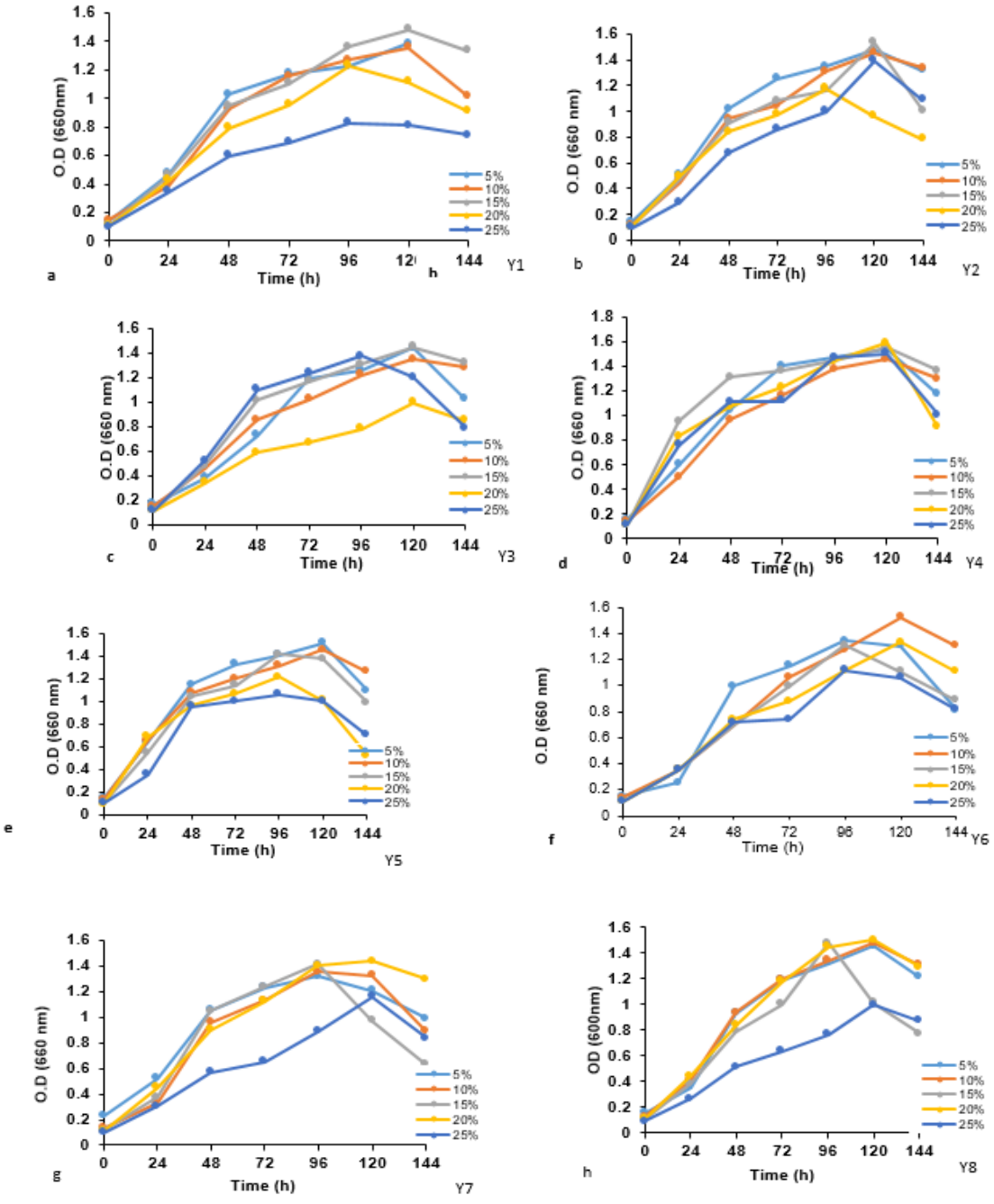


**Figure 3:** (a-h): Growth profile of yeast strain Y1-Y8 at different glucose concentration. Values are mean (n=3) ± SD. Error bar appearing below marker are not visible

*3.6 Molecular characterization yeast strain Y4*

By examining the molecular sequence of the ITS1 and ITS4 and the LR0R and LR7 domains, it was possible to identify the yeast strain Y4, the best isolate from the stress-exclusive test (ethanol, glucose, and temperature tolerance). When the nucleotide sequences

were compared to those from the NCBI, *Kazachstania pseudohumilis* had the highest similarity index (96.07%). According to the phylogenetic tree presented (Figure 4), Yeast strain Y4 was revealed to be closely related to specie of *K. pseudohumilis* GCMCC 2.3956. Hence, identified as *Kazachstania pseudohumilis* Y4. The genomic sequence was deposited with accession number, ON876535:1. *K. pseudohumilis, previously known as Candida pseudohumilis* was recently reassigned to the genus *Kazachstania* based on the new code of fungi nomenclature [49]. *Kazachstania* sp. have been identified as a sough dough yeast [50]. Similarly, Tadesse, et al. [51], identified *Kazachstania bulderi* among the yeast responsible for *Injera* fermentation. Species of *Kazachstania* existed in consortium with some LAB species in sough dough due to mutual relationship [52].

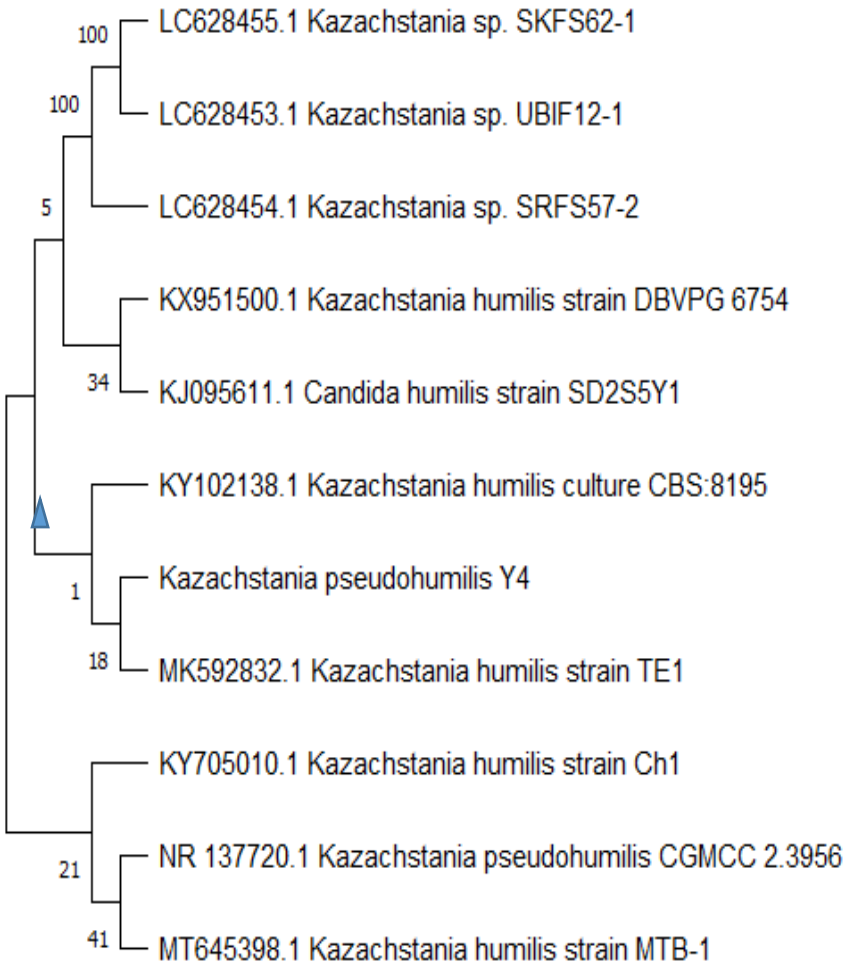


**Figure 4:** Phylogenetic –Neighbor joining analysis tree for relatedness of yeast strain Y4 using maximum likelihood analysis on MEGA 7.0 software. Frequencies with which a branch appears in bootstrap replication is indicated by the numbers on the branches

The *K. humilis* species was discovered to be prevalent during the development of maize-unsalable vegetable silage [53]. In another study, Punyauppa-path, et al. [54], found that 33.0% of the yeast they isolated from fermented foods from northeastern Thailand was *Kazachstania* sp. *K. marxianus* was also discovered by Johansen, et al. [55], in fermented foods with a grain foundation that originated in Sub-Saharan Africa. According to the literature search, this work is the first to describe *K. pseudohumilis* Y 4 which was isolated from fermented food in Nigeria.

## Conclusion

Nigerian local food waste represents a viable supply of fermenting yeast with great industrial value. This work effectively isolated a creamy, opaque, butyrous, budding *Kazachstania pseudohumilis* Y4 from a Nigerian fermented food waste known as "fufu." When compared to other isolated strains, this strain showed a higher level of ethanol tolerance. The strain's ability to withstand heat has demonstrated its distinctiveness. As a result of *K. pseudohumilis* Y4's great tolerance to high starting glucose concentration, it may be suited for high glucose conversion rates, which are needed in the bioethanol sector. For potential bioethanol production from food waste, *Kazachstania pseudohumilis* Y4 is interesting and merits further study. Furthermore, this is the first time that this strain of indigenous fermented food from Nigeria has been reported.

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## Conflict of interest

Conflict of interest: Authors declared that there is no conflict of interest

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