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Optimization and Characterization of Alcohol Production from Agro-Wastes by Indigenous Candida tropicalis Strain IDRI000028827 and Commercial Saccharomyces cerevisiae

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Abstract: Alcohol is widely used in Nigeria across different industries, but its production is costly because it depends on commercial yeast (Saccharomyces cerevisiae) and refined substrates. This study investigated the use of agro-wastes for alcohol production using an indigenous yeast strain, Candida tropicalis IDRI000028827, and compared its performance with S. cerevisiae under similar conditions. Agro-waste substrates were prepared from date palm, corn, sugarcane, pineapple, and banana (carbon sources), and from defatted groundnut, bambara nut waste, crayfish by-products, eggshell, and snail shell (nitrogen sources). The materials were dried, powdered, and subjected to fermentation under varying temperatures (15–40 °C) and pH values (4.0–7.0). Alcohol was distilled, quantified, and analyzed by Gas Chromatography–Mass Spectrometry (GC–MS). Antibacterial activity of the alcohol was tested against Staphylococcus aureus OPD001-1, Bacillus sp. FFOS, and Salmonella enterica strains 2011k-1440 and CFSANO27396.Results showed that S. cerevisiae produced higher yields (116.7%) under refined substrates, while C. tropicalis performed better on agro-wastes. Optimal yields were obtained from sugarcane bagasse (3.3% w/v) and bambara nut waste (3.1% w/v) at 25 °C and pH 5.5. Alcohol yield from C. tropicalis with agro waste (66.7% w/v) was significantly higher than from S. cerevisiae (15.3% w/v) ($p < 0.05$). GC–MS confirmed that C. tropicalis generated higher levels of methanol (5.63 ppm), ethanol (4.40 ppm), and propanol (3.15 ppm), compared with S. cerevisiae. The alcohol from C. tropicalis also showed stronger antibacterial activity (80–92%) than that from S. cerevisiae (35–58%).This study highlights C. tropicalis IDRI000028827 as a promising low-cost alternative for bio-alcohol production, with sugarcane bagasse and bambara nut waste serving as efficient, sustainable substrates.

Key words: Agro-wastes, Optimization, Alcohol production, Characterization, Candida tropicalis IDRI000028827, Saccharomyces cerevisiae

Introduction

Over the centuries, fossil fuels along with synthetic alcohols derived from them have served as major sources of energy. Yet, their adverse environmental effects have intensified the global search for greener alternatives. Increasingly, researchers are exploring biological processes as sustainable options. In West Africa, particularly Nigeria, Saccharomyces cerevisiae has traditionally been the primary yeast used in alcohol production, often relying on refined and costly substrates (Onyema et al., 2023). However, the reliance on expensive inocula and imported substrates worsened because of the fluctuating currency exchange rates which pose serious economic challenges for local industries. Moreover, diverting edible crops to fermentation as a substitute raises the risk of food insecurity, adding further pressure to already vulnerable populations (Ahamefule et al., 2024).An attractive alternative lies in the use of rich food wastes, which are abundant, affordable, and environmentally friendly (Shah et al., 2025; Ngando-Same et al., 2024). Unfortunately, conventional S. cerevisiae strains have limited ability to hydrolyze such complex wastes effectively (Li et al., 2022), restricting alcohol yield and productivity. This highlights the need for alternative microorganism with greater hydrolytic capacity and tolerance to osmotic and fermentation stresses. Non-conventional yeasts such as Candida tropicalis have recently gained attention for their versatility in fermenting carbohydrate-based wastes into diverse metabolites. Unlike S. cerevisiae, C. tropicalis can utilize both hexose and pentose sugars while withstanding toxic by-products generated during fermentation (Ayepa et al., 2024). Its potential in processing agricultural rCVesidues has been documented in several studies (Sorour et al., 2023; Shariq and Sohail, 2019; Hermansyah et al., 2015). However, limited research has focused on its efficiency in converting locally available agro-wastes into alcohols relative to commercial S. cerevisiae strains under optimized fermentation conditions, or on assessing its alcohol for antimicrobial properties. This study therefore set out to address that gap by optimizing and evaluating alcohol production from agro-wastes using an indigenous strain, C. tropicalis IDRI000028827, and benchmarking its performance against commercial S. cerevisiae. The outcomes are expected to support the use of this local strain for cost-effective alcohol fermentation, offering scalable solutions particularly suited for resource-limited economic countries such as Nigeria.

Materials and Methods

Microorganism

The test organism used in this study was C. tropicalis strain IDRI000028827, previously reported to possess alcohol tolerance (Onyia et al., 2025). The strain was obtained from the Culture Collection Laboratory of the Department of Microbiology, Enugu State University of Science and Technology. Its stock culture was maintained

in glycerol stock on Potato Dextrose agar (PDA) at 4 °C until when required for experimentation.

Substrate Collection and Preparation

Carbon sources including date palm waste, corn waste, sugarcane bagasse, pineapple peels, and banana peels were collected from different processing sites within Enugu metropolis. Nitrogen sources such as defatted groundnut, bambara nut waste, crayfish processing by-products, eggshells, and snail shells were also sourced locally. All collected substrates were rinsed thoroughly with sterile water to remove dirt and impurities. The materials were then sun-dried at ambient temperature, ground into powder using a manual home blender (Corona), and sieved through muslin cloth to obtain uniform particle size suitable for fermentation.

Activation of C. tropicalis IDRI000028827 Culture

The yeast preserved in glycerol stock was first streaked onto Potato Dextrose Agar (PDA) plates and incubated at 30 °C for 48 hours to obtain actively growing colonies. The resulting biomass was used as inoculum for fermentation experiments.

Preliminary Alcohol Fermentation Using Refined Substrates

The preliminary alcohol production by C. tropicalis and S. cerevisiae was carried out with refined substrates in 500 ml Erlenmeyer flasks containing sterile Mineral Salt medium composed of glucose (2.5% w/v), yeast extract (1.5 % w/v), NH_4SO_4(0.5 % w/v), KH_2PO_4 (0.1% w/v), and $MgCl_2.6H_2O$ (0.05% w/v) prior to optimization studies. The medium was sterilized at 121 °C for 15 min prior to inoculation. The activated yeast inoculum (0.3% w/v) was introduced aseptically into the fermentation medium, and incubation was performed at 30 °C for 7 days under stationary (anaerobic) conditions (Eeomet al. (2018)).

Distillation of the Alcohol

The harvested fermented broth was centrifuged (Eppendorf, Germany) at 1000rpm and later subjected to distillation using a laboratory distiller (Spaqlabs, India) to recover the alcohol. The alcohol content was then quantified using a refractometer following the procedure of Gerogiannaki-Christopoulouet al. (2003). Prior to measurement, the refractometer was calibrated to 0% alcohol at room temperature. A drop of the distilled sample was placed on the prism surface, and the reading was recorded. The percentage alcohol content was calculated using the following formula:

Alcohol content (%) =Refractive index of test alcohol- Refractive index of water X 100

Refractive index of pure alcohol (Control) - Refractive index of water

Where
Refractive index of water =0 and
Refractive index of pure alcohol (ethanol) =3

Optimization Study with Indigenous Agro-Wastes

i. Substrate Screening

The one-factor-at-a-time (OFAT) approach, as adopted by George-Okafor and Mike-Anosike (2012), was used for substrate screening. In this method, each carbon source (2.5% w/v) was used to replace glucose in earlier outlined fermentation medium, with an inoculum concentration of 0.3%. Anaerobic static fermentation was performed at 30 °C for 7 days, following the procedure of Eeomet al. (2018). The same procedure was applied for nitrogen sources, where yeast extract was replaced with each test nitrogen substrate at a concentration of 1.5% (w/v).The carbon and nitrogen sources that produced the highest alcohol yields were selected for further analysis. Their concentration ranges were tested from 0.9% to 3.7% for carbon substrates and from 0.7% to 3.9%for nitrogen substrates to determine optimal concentrations. The concentrations that resulted in the highest alcohol production were chosen for subsequent optimization experiments.

ii. Fermentation Condition Profile

The effect of temperature on alcohol production was evaluated by carrying out anaerobic fermentation with the selected substrates at temperatures between 15 °C and 40 °C for 7 days. Similarly, the influence of pH was assessed by adjusting the fermentation medium within a range of pH 4.0–7.0 under anaerobic conditions at the previously optimized temperature for 7 days. All optimization experiments were also conducted with a commercial strain of S. cerevisiae, which served as the control.

Optimal Alcohol Production Using Agro-Wastes

Both the test organism **(**C. tropicalis Strain IDRI000028827and S. cerevisiae) were subjected to a larger scale alcohol fermentation (2.5 litres) by inoculating0.3% biomass into fermentation medium composed of sugarcane bagasse (3.3%) bambara nut waste (3.1%), NH_4SO_4(0.5%), KH_2PO_4 (0.1%), and $MgCl_2.6H_2O$ (0.05%) fermentation under optimized conditionat pH of 5.5, and temp of 25°C for 7days. The produced crude alcohol was distilled as earlier described.

Characterization of Distilled Alcohol

The type, quantity, and antibacterial properties of alcohol produced by C. tropicalis strain IDRI000028827 and S. cerevisiae were determined and compared.

Alcohol Profiling and Quantification

The quantification of the type of alcohol produced was carried out using the Gas Chromatography–Mass Spectrometry (GC–MS) technique described by Saito (1996).

For each sample, 1.5 ml of alcohol was filtered into vials using 0.45 µm cellulose acetate micro filters. A solution of 5 ml acetonitrile was transferred into a graduated vial and diluted to 10 ml with the filtered sample. The acetonitrile layer was concentrated to 1 ml, and 100 µL was injected into the GC-FID. The analysis was performed using an Agilent 6590 gas chromatograph fitted with a polyethylene glycol TPA-modified column (ZB-FFAP, 30 m × 0.32 mm. × 0.25 µm; Chrompak, Middleburg, Netherlands) and a flame ionization detector. A split/splitless injector was employed, with both injection and detection temperatures maintained at 300 °C. Nitrogen served as the carrier gas at a flow rate of 2 ml/min. Then the initial column temperature was set at 35 °C and held for 3 min. The temperature was then ramped to: 40 °C at 2.5 °C min–1 and held for 2 min; 80 °C at 20 °C min–1 and held for 4 min; 140 °C at 20 °C min–1 and held for 5 min; 220 °C at 20 °C min–1 with a final hold of 1 min. The total run time was 26 min. Calibration curves were generated from peak area ratios of known standards at different concentrations compared to an internal standard. Working standards were prepared by serial dilutions (0.5–2.0 µg/dl) in 40% acetonitrile. Unknown compounds in the samples were identified by matching their retention times with those of the standards.

Antibacterial Evaluation of the Agro-Waste- Alcohol

The antibacterial activity of alcohol generated by C. tropicalis strain IDRI000028827 was tested against four bacterial pathogens: Salmonella enterica 2011k-1440, S. enterica serovar CFSAN027396, Staphylococcus aureus OPD001-1, and Bacillus subtilis FFOS. Each strain was prepared in sterile saline and adjusted to McFarland's standard to obtain a final concentration of ~1.0×10^8 CFU/ml. For the assay, 3 ml of bacterial suspension was combined with 3 ml of test alcohol (70% v/v) in sterile tubes and kept at room temperature for 30 minutes without agitation. The mixtures were then transferred into sterile beakers and gently agitated in a water bath at 37 °C to remove residual alcohol. Subsequently, 1 ml of each treated sample was plated using the pour plate method. Selective media were used: Salmonella-Shigella agar for Salmonella, Mannitol Salt Agar for S. aureus, and Nutrient Agar for B. subtilis. The same procedure was repeated using agro-waste-alcohol produced by S. cerevisiae. Ethanol from refined substrates served as a positive control, while samples without alcohol served as the negative control. Plates were incubated at 37 °C for 24 h, and colonies were counted using a digital colony counter (Equitronmodel 0671M(GMP/desk-top digital model, India). Antibacterial activity was expressed as percentage inhibition, calculated from survival values relative to the negative control using the formular:

$$\% \text{ survival} = \frac{\text{Colony Forming Unit in Treated Samples}}{\text{Colony Forming Unit in Negative control}} \times 100\%$$

Then % Inhibition =100%−% survival

Where 100% represented plate without any growth

Statistical Analysis

All experimental data were analyzed using IBM SPSS Statistics software, version 18. Results were expressed as mean ± standard deviation. One-way analysis of variance (A One-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons was applied to assess differences across groups. A probability value of $p < 0.05$ was considered statistically significant for both within- and between-group comparisons.

Results and Discussion

Alcohol Yields from Refined Fermentation Medium

The preliminary alcohol production carried out with refined substrates revealed that S. cerevisiae produced 116.7% alcohol, while C. tropicalis produced only 86.7% alcohol. This can be explained with the fact that most refined substrates are hexoses sugar (e.g., glucose, sucrose) which S. cerevisiae preferentially metabolized than pentoses even under unfavourable condition. S. cerevisiae is known to possess highly active enzymes such as alcohol dehydrogenase and pyruvate decarboxylase that convert glucose to ethanol more efficiently than in most non-Saccharomyces yeasts (Walker, 2011) It can also continue fermenting even when alcohol levels become toxic to other microorganisms. In contrast, C. tropical is sugar metabolism slows down, under anaerobic condition and part of it becomes converted into by-products like organic acids and glycerol instead of ethanol (Barnett, 2003). Generally, the difference in alcohol yield also reflects variations in substrate utilization and cellular adaptation.

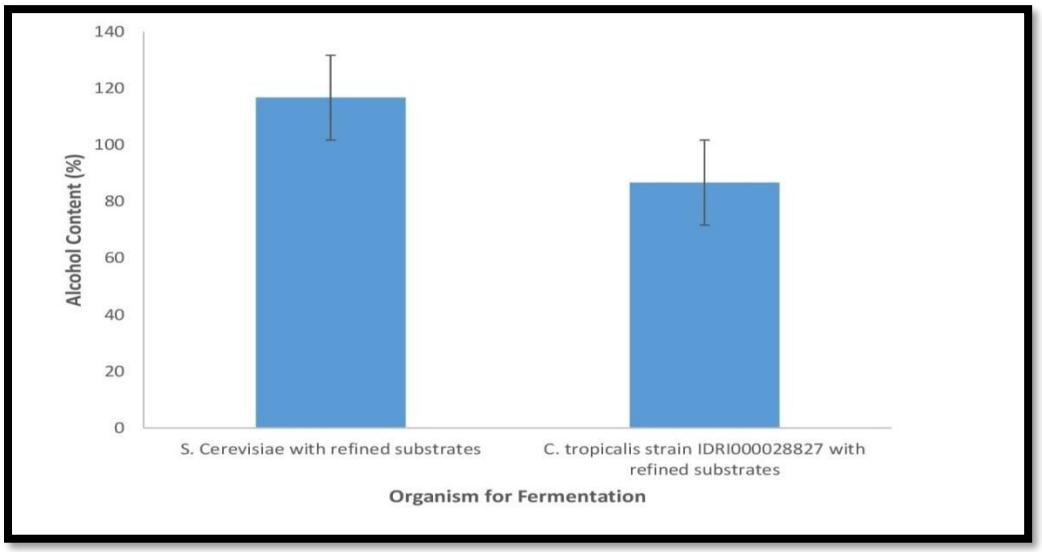

Fig 1. Alcohol yield of Candida tropicalis and Saccharomyces cerevisiae on refined substrates

Carbon Substrate Profile

Sugarcane bagasse supported the highest alcohol yield (66.7% w/v) at an optimum concentration of 3.3% (Fig. 2a–b). This agrees with previous reports highlighting sugarcane bagasse as an efficient substrate for proteolytic enzyme and bioethanol production (George-Okafor and Mike-Anosike, 2012; Jeyaram et al., 2024). Its effectiveness is likely due to its fermentable carbohydrate content, which drives glycolysis and alcohol formation. Although You et al. (2017) reported optimal yields at 5% pretreated bagasse, the lower concentration observed in this study may reflect the absence of pretreatment, which otherwise enhances substrate accessibility and minimizes fermentation inhibition (Sun et al., 2016).

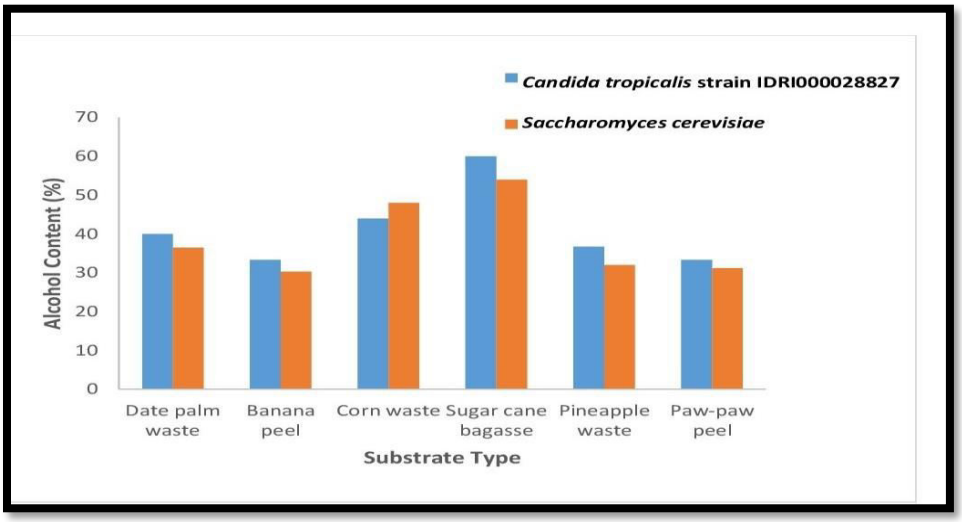

Figure 2a: Effect of agro-wastes carbon on alcohol production by C. tropicalis strain IDRI000028827 and commercial Saccharomyces cerevisiae

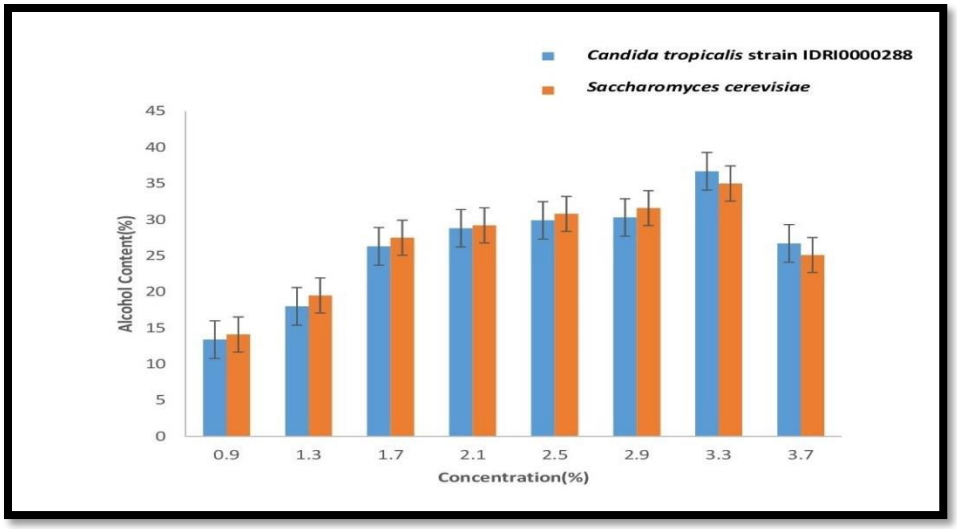

Figure 2b: Effect of concentration of sugarcane bagasse on alcohol yield by C. tropicalis strain IDRI0000288 and S. cerevisiae

Nitrogen Source

For C. tropicalis, the highest alcohol production was observed with bambara nut waste (83.3%), followed by snail shell (70%) and crayfish waste (66.7%) (Fig. 3a). This observation is consistent with earlier reports highlighting the value of bambara nut residues in industrial fermentations (Lungaho et al., 2025). The improved yield compared with the initial level (66.3%, Fig. 2a) can be attributed to the typical carbon-to-nitrogen balance, where limited nitrogen availability promotes alcohol formation, whereas excess nitrogen generally enhances cell growth. At higher substrate concentrations (3.5–3.9%, Fig. 3b), yields dropped sharply, most likely as a result of substrate inhibition, osmotic stress, or the accumulation of toxic metabolites; this reduction was more severe in S. cerevisiae. Conversely, at lower substrate levels (0.7–1.9%), both yeasts produced only moderate alcohol levels, indicating possible restriction by fermentable sugar or nutrient availability. Overall, the results suggest that while both organisms are capable of fermenting bambara nut waste, C. tropicalis demonstrated a slight advantage in both alcohol yield and tolerance at elevated substrate levels. However, the heterogeneous nature of bambara nut waste could havelimited sugar accessibility and introduced inhibitory factors that compromised yeast performance (Sun et al., 2016).

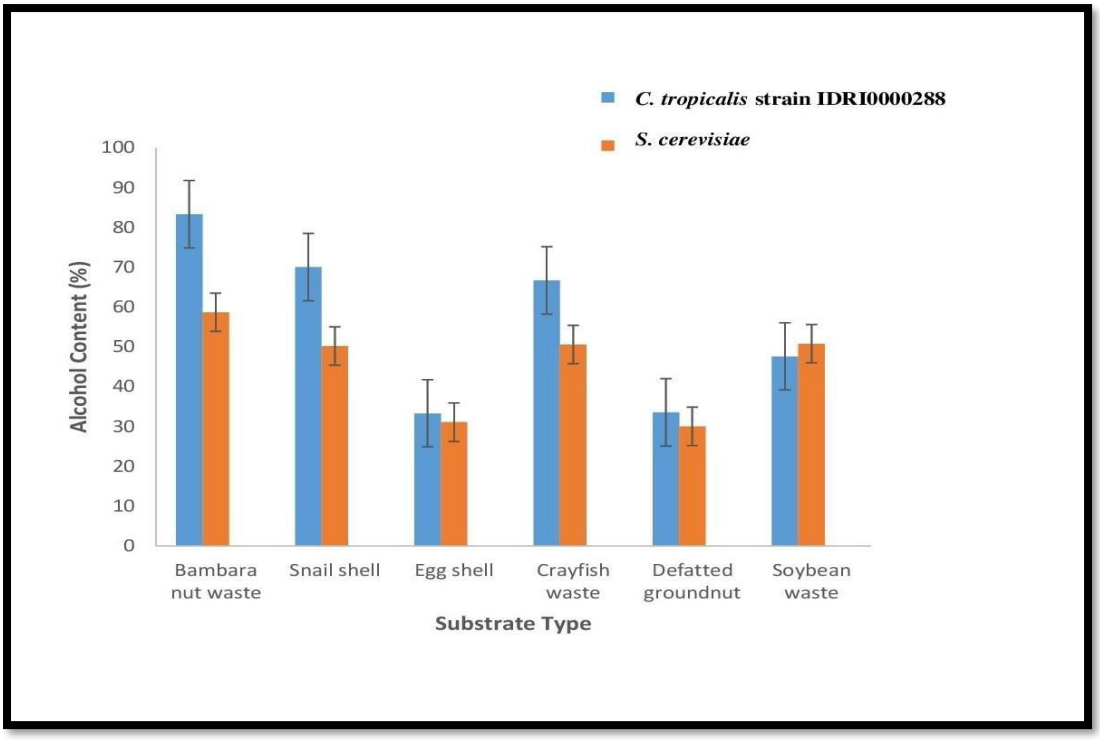

Figure 3a: Effect of agro waste nitrogen content on alcohol production by Candida tropicalis strain IDRI000028827 and S. cerevisiae

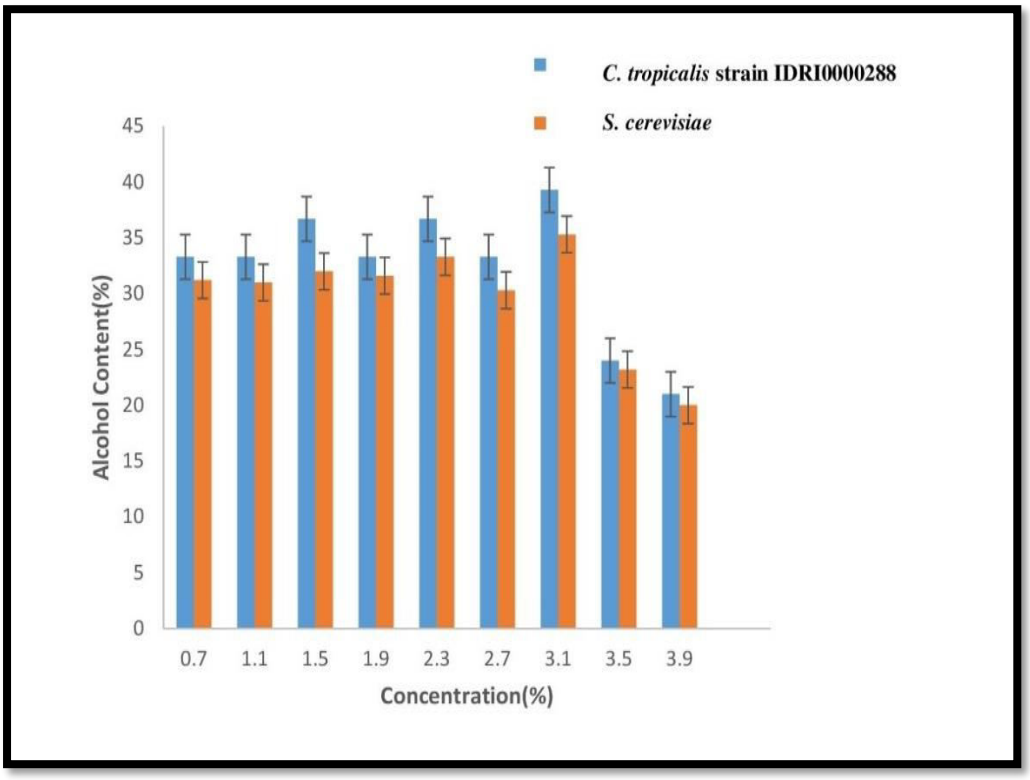

Figure 3b: Concentration profile of bambara nut waste on alcohol yield by Candida tropicalis strain IDRI000028827 and S. cerevisiae

Temperature Profile

C. tropicalis strain IDRI000028827 produced maximum alcohol at 25 °C (Fig. 4). This optimum temperature is consistent with earlier findings reporting peak alcohol production by C. tropicalis (YMEC14) at 25 °C (Jamai et al., 2001), but differs from the 30 °C optimum reported by Zulfikar et al. (2019) for C. tropicalis (JCB1-24). In general, yeasts, including the conventional S. cerevisiae, achieved maximum alcohol production within the mesophilic range (20–40 °C), where enzyme activity, membrane stability, cell growth, and ethanol tolerance are often enhanced (Liszkowska and Berlowska , 2021).

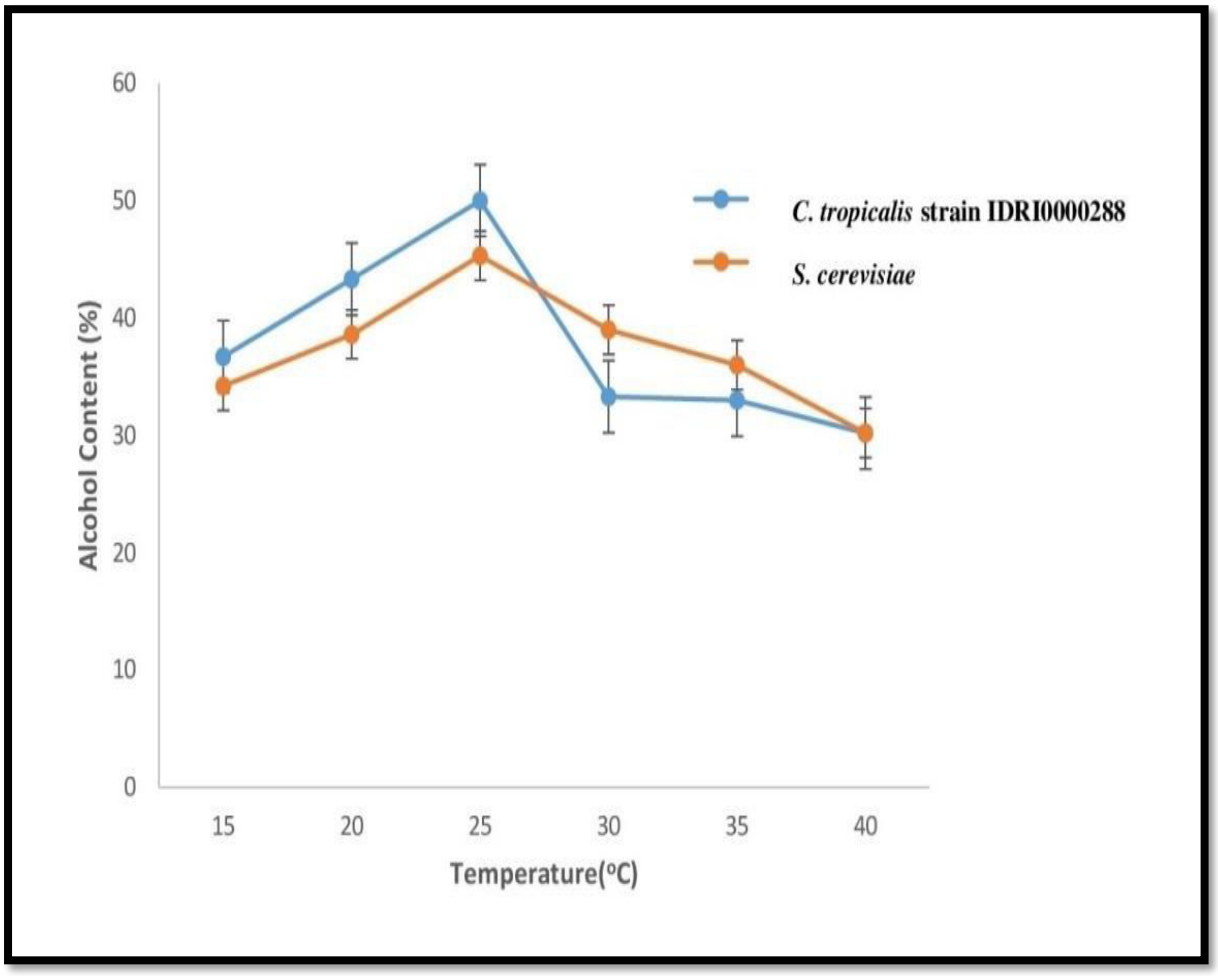

Fig. 4: Effect of temperature on alcohol production by C. tropicalis strain IDRI000028827 and S. cerevisiae

pH Profile

Maximum alcohol production was achieved at pH 5.5 by C. tropicalis strain IDRI000028827(40%) and S. cerevisiae (37.8%) while yields declined with increasing pH, reaching 10% at pH 7 (Fig. 5). This finding is consistent with previous reports indicating that most yeasts produce alcohol optimally within pH 4.5–5.5 (Sopandi and Wardah, 2017). Similarly, agro-waste fermentations have been shown to support higher yields in this range (Hashem et al., 2021). The observed optimum of pH 5.5 for C. tropicalis strain IDRI000028827 and S. cerevisiae therefore aligns with established patterns, as yeast enzymes are more metabolically active under these conditions, while the slightly acidic environment also minimizes microbial contamination.

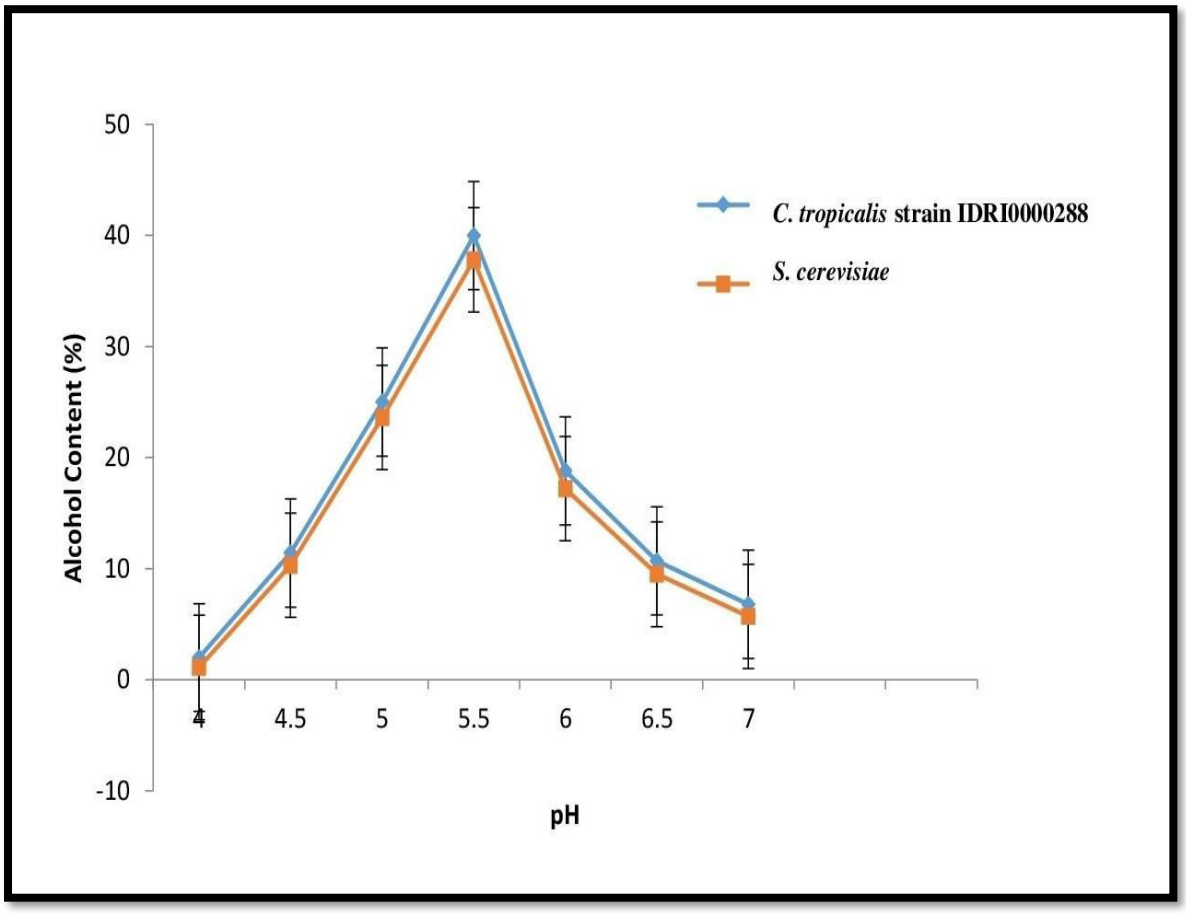

Fig. 5: Effect of pH on alcohol production by Candida tropicalis strain IDRI000028827 and S. cerevisiae

Alcohol Yields with Agro-Wastes Under Optimized Conditions

Scale-up production (2.5litres) under optimized condition (3.3% sugarcane bagasse, Bambara nut wastes, at 25 °C, pH 5.5, for 7 days), C tropicalis strain IDRI000028827 produced 66.7% alcohol, compared with 15.3% by the commercial S. cerevisiae (Fig. 6). This disparity highlights the limited efficiency of commercial S. cerevisiae in fermenting complex agro-waste substrates. Previous studies have linked such limitations to its restricted ability to metabolize pentose sugars, lower tolerance to inhibitory compounds, reduced stress resilience, and narrower substrate utilization range (Maltam et al., 2016; Jamaluddin et al., 2023). Furthermore, its relatively poor performance may also reflect slower adaptation to indigenous waste materials, in contrast to C. tropicalis strain IDRI000028827, which was locally isolated from orange juice and could therefore possessed higher adaptability to native substrates (Onyia et al., 2025).

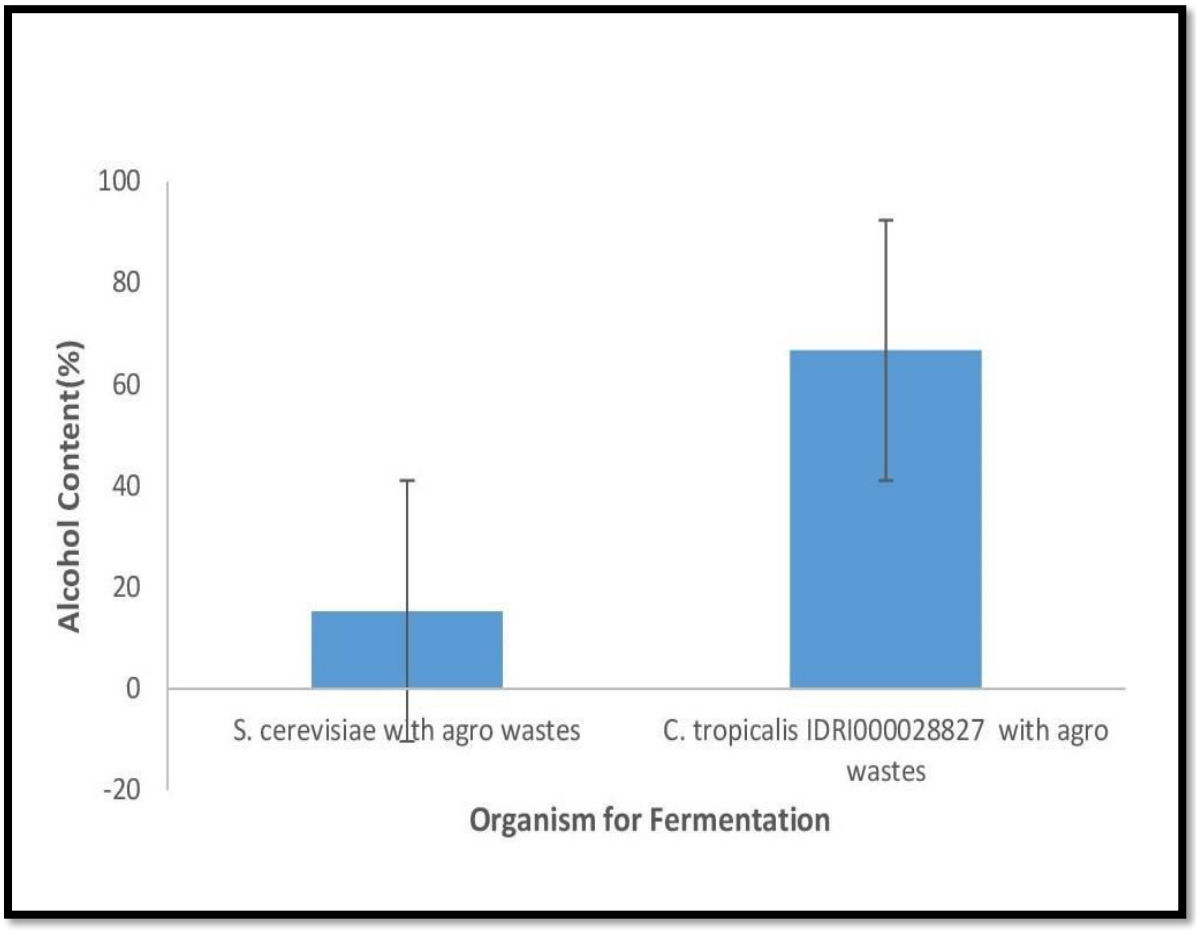

Fig. 6: Alcohol produced with agrowastes by C. tropicalis strain IDRI000028827 and S. cerevisiae under optimized condition

Alcohol Identification and Quantification

Gas Chromatography–Mass Spectrometry (GC–MS) analysis confirmed the presence of methanol, ethanol, propanol and butanol in the fermented broth of both the test and standard yeast strains (Figs. 7a–b). Candida tropicalis strain IDRI000028827 produced a significantly higher amount of ethanol (4.40 ppm, $P<0.05$) compared with S. cerevisiae (1.72ppm) for fermentation with agrowastes. Ethanol production by C. tropicalis has previously been documented for strains ATCC 750 and 4kr27 (Liu et al., 2019; Zulfikar et al., 2019). The detection of propanol in this study is also consistent with findings by Singh et al. (2023), who reported its production alongside ethanol when C. tropicalis (K21D) was cultivated on different carbon sources. The comparatively higher production of multiple alcohol types by strain IDRI000028827, relative to commercial S. cerevisiae under the same conditions, indicates its potential for utilization. This capacity suggests promising applications in waste-to-biofuel conversion and highlights the need for additional optimization and waste treatment studies to maximize yield.

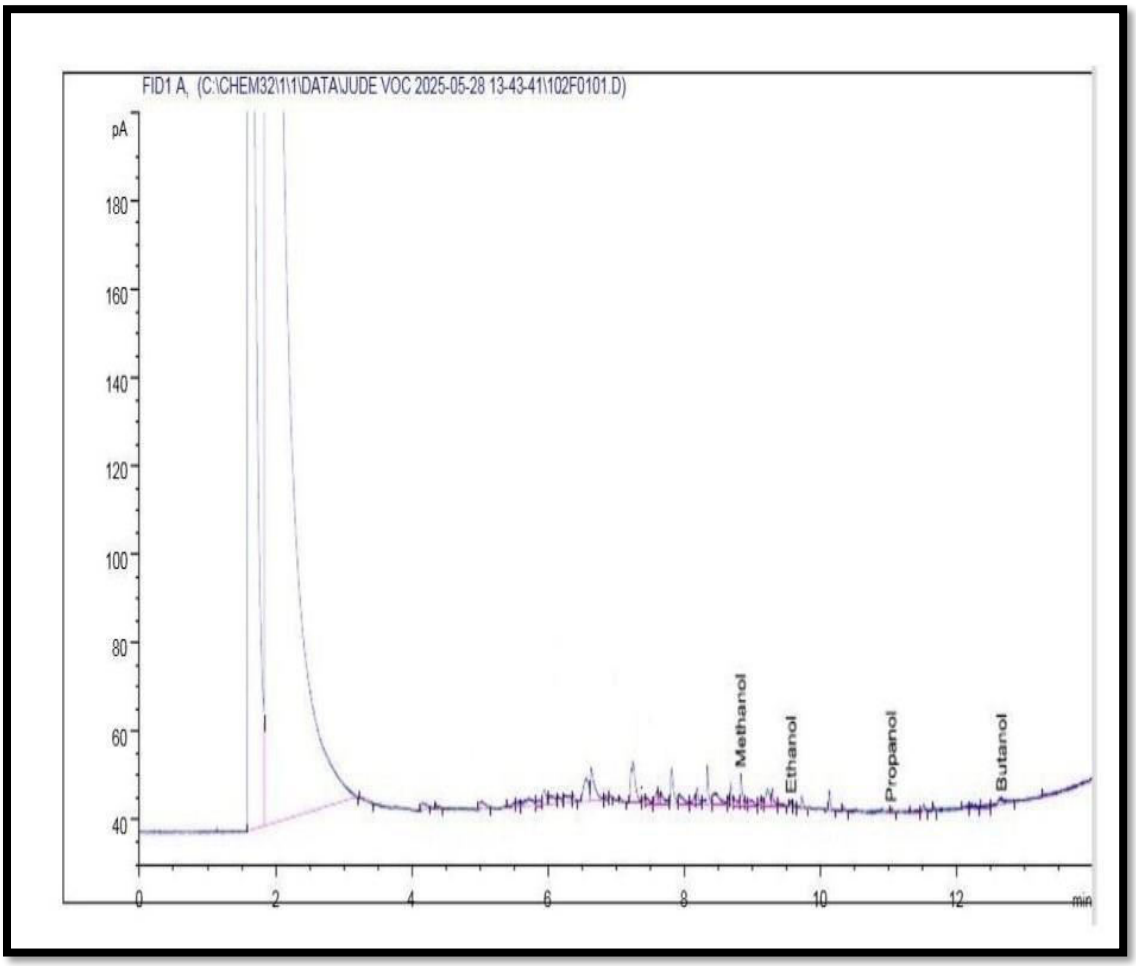

Fig. 7a: Chromatogram of agrowaste-alcohol produced by C. tropicalis strain IDRI000028827.

Key:

Name: Candida tropcalis strain IDRI000028827

Ret Time [min]	Type	Area [pA*s]	Amt/Area	Amount [ppm)	Grp	Name
9.433	BB	20.13767	7.76010e-1	5.62704	1	Methanol
13.178	VV	5.70060	7.72667e-1	4.40466	1	Ethanol
14.434	VV	68.42715	7.76764e-1	3.15172	1	Propanol
14.584	VB	13.65857	7.75237e-1	0.58862	1	Butanol

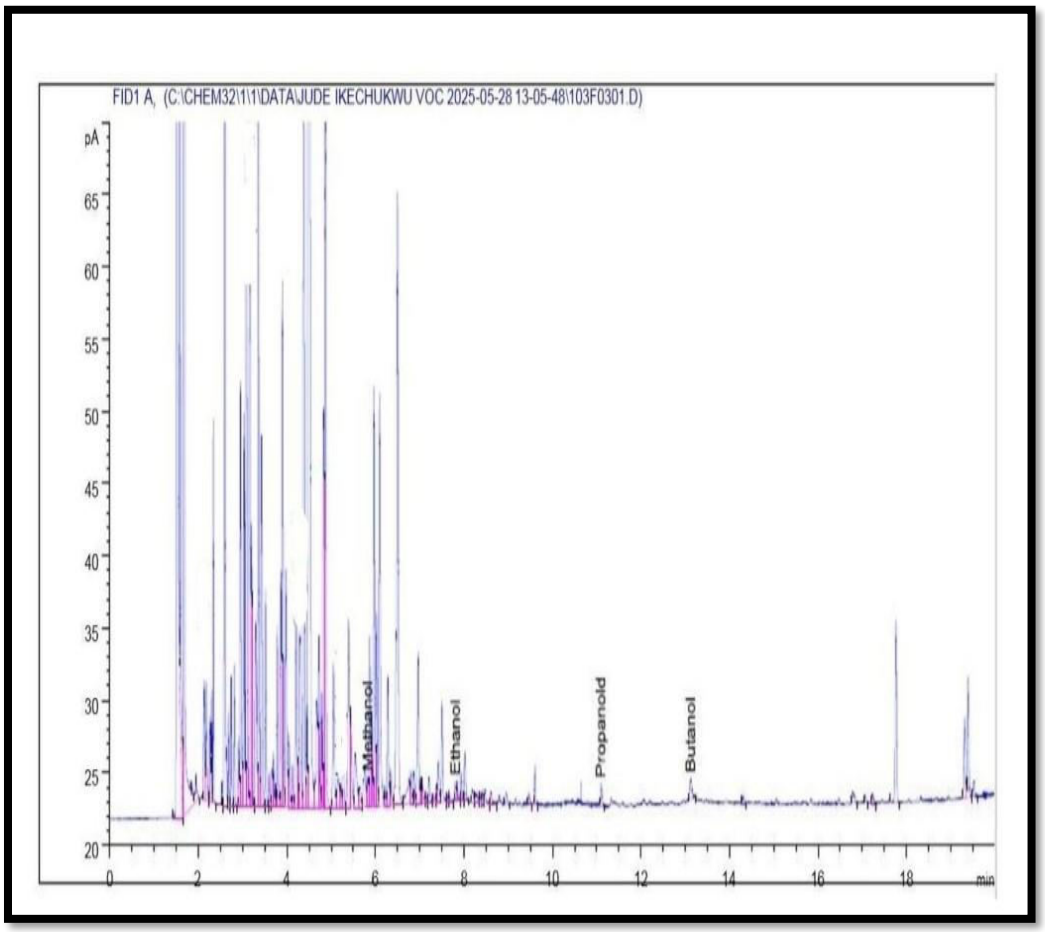

Fig.7b: Chromatogram of agrowaste-alcohol types produced by S. cerevisiae

Key:

Sample Name: Saccharomyces cerevisiae

Ret Time [min]	Type	Area [pA*s]	Amt/Area	Amount [ppm)	Grp	Name
5.835	VV	4.31365	7.71514e-1	3.32804	1	Methanol
7.821	BV	2.25051	7.65632e-1	1.72306	1	Ethanol
11.112	BB	2.81797	7.67891e-1	2.16389	1	Propanol
13.132	BB	4.91858	7.71807e-1	3.79619	1	Butanol

Antibacterial Efficacy of Produced Alcohol

The antimicrobial performance of alcohol produced by C. tropicalis and S. cerevisiae on agro-waste substrates was evaluated against a refined-substrate alcohol (control). As expected, ethanol obtained from S. cerevisiae cultivated on refined substrates demonstrated the highest activity, completely inhibiting both strains of Salmonella enterica and Staphylococcus aureus, and nearly eliminating Bacillus subtilis. This aligns with earlier reports attributing the strong antimicrobial effect of pure ethanol to its ability to denature proteins and disrupt microbial membranes (Mathew,2024;Kampf, 2018).A marked reduction in inhibition was observed with S. cerevisiae agro-waste alcohol, with activity ranging between 35% and 58%. This decline suggests reduced ethanol yield and purity, possibly due to limited fermentable sugars in the waste material or the presence of inhibitory fermentation by-products (Demiray et al., 2018). The relatively stronger activity against S. aureus compared to Salmonella reflects the higher susceptibility of Gram-positive organisms to alcohol, consistent with differences in cell wall architecture (Russell, 2003). Generally, agro-waste alcohol derived from C. tropicalis retained substantially higher activity, with inhibition values of 80–92% across all pathogens. Its performance against B. subtilis (92%) and S. aureus (90%) approached that of the control, while inhibition of Salmonella strains (80–90%) also remained high. Comparatively, agro-waste-alcohol from C. tropicalis IDR1000028827 significantly inhibited all the assayed test organisms more than S. cerevisiae with agro- waste at $p< 0.01$. This positions C. tropicalis alcohol as a strongbio-disinfectant and preservative, alternative to synthetic ethanol for application

Table 4.3: Percentage Inhibition of the Produced Alcohol against Test Pathogens

	% Alcohol Inhibition From		
Test Organism	**S. cerevisiae with Refined Substrates (Control)**	**S. cerevisiae with Agro- waste (Test)**	**C. tropicalis IDR1000028827 with Agro-wastes (Test)**
Salmonella enterica 2011k-1440	*100	35	90
Salmonella enteric subspenterica sesovar CFSANO27396	100	49	80
Staphylococcus aureus OPD001-1	100	58	90
Bacillus subtilis FFOS	98	55	92

*100% was assigned to plates without organisms after treatment and used to calculate the inhibition rate of the test alcohol.

Conclusion

The findings of this study highlight the efficiency of C. tropicalis strain IDRI000028827 in converting agro-wastes such as sugarcane bagasse and bambara nut residues into alcohol. Compared with commercial S. cerevisiae, the strain produced higher alcohol yields, generated a wider range of alcohols, and showed stronger antibacterial effects. These results suggest its promise as a sustainable and cost-effective option for industrial alcohol production, reducing dependence on refined substrates and imported yeast.

References

1. Ahamefule, C. S., Osilo, C., Ahamefule, B. C., Madueke, S. N. and Moneke, A. N. (2024). Current Research in Microbial Sciences, Simultaneous production of biofuel from agricultural wastes and bioremediation of the waste substrates: A review. Volume 7, 100305.
2. Ayepa, E., Li, Q., Zhang, Z., Wang, H., Herman, R. A., Ouyang, Y., Kuang, X., Tafere, G. A. and Ma, M. (2024).Biomass and Bioenergy, Molecular mechanism of tolerance evolution in Candida tropicalis to inhibitory compounds derived from corn stover hydrolysis. Volume 181, 107031.
3. Barnett, J. A. (2003).Microbiology, Beginnings of microbiology and biochemistry: The contribution of yeast research. Volume 149 Number 3: Page. 557–567.
4. Demiray, E., Karatay, S. and Dönmez, G. (2018). Energy, Evaluation of pomegranate peel in ethanol production by Saccharomyces cerevisiae and Pichia stipites.Volume 159.
5. Eeom, Y. J., Son, S. Y., Jung, D. H., Hur, M. S., Kim, C. M., Park, S. Y., Shin, W. C., Lee, S. J., Auh, J. H., Kim, G. W. and Park, C. S. (2018).Food Science and Biotechnology, Diversity analysis of Saccharomyces cerevisiae isolated from natural sources by multilocus sequence typing (MLST). Volume 27 Number 4: Page. 1119–1127.
6. George-Okafor, U.O. and Mike-Anosike, E.E. (2012). Research Journal of Microbiology, Screening and Optimal Protease Production by Bacillus sp. Sw-2 Using Low Cost Substrate Medium. Volume 7: Page. 327-336
7. Gerogiannaki-Christopoulou, M., Kyriakidis, N. V. and Athanasopoulos, P. E. (2003). Journal of AOAC International, New refractive index method for measurement of alcoholic strength of small volume samples. Volume 86 Number 6: Page. 1232–1235.
8. Hashem, M., Abu-Elreesh, G. and Abdel-Hamid, A. M. (2021). Journal of Environmental Chemical Engineering, Bioethanol production from agricultural

wastes by Candida tropicalis under optimized fermentation conditions. Volume 9 Number 5: Page. 105–118.

9. Hermansyah, H., Novia, N., Sugiyama, M. and Harashima, S. (2015).Microbiology and Biotechnology Letters, Candida tropicalis isolated from Tuak, a North Sumatera-Indonesian traditional beverage, for bioethanol production. Volume 43 Number 3: Page. 241–248.
10. Jamai, L., Sendide, K., Ettayebi, K., Errachidi, F., Hamdouni-Alami, O., Tahri-Jouti, M. A., Mc Dermott, T. and Ettayebi, M. (2001). Physiological difference during ethanol fermentation between calcium alginate-immobilized Candida tropicalis and Saccharomyces cerevisiae. Volume 204 Number 2: 375–379.
11. Jamaluddin, A., Riyanti, E.I., Mubarik, N.R. and Listanto, E. (2023). Tropical Life Sciences Research, "Construction of Novel Yeast Strains from Candida tropicalis KBKTI 10.5.1 and Saccharomyces cerevisiae DBY1 to improve the performance of ethanol production using lignocellulosic hydrolysate."Volume 34 Number 2: Page. 81-107.
12. Jeyaram, K., Devi, T. R. and Singh, M. R. (2024). Bioresource Technology Reports, Valorization of sugarcane bagasse for bioethanol production using optimized fermentation processes. Volume 26, 101625.
13. Kampf, G. (2018). Journal of Hospital Infection, Efficacy of ethanol against viruses in hand disinfection. Volume 98: Page. 331–338.
14. Li, B., Liu, N. and Zhao, X. (2022).Biotechnology for Biofuels and Bioproducts, Response mechanisms of Saccharomyces cerevisiae to the stress factors present in lignocellulose hydrolysate and strategies for constructing robust strains. Volume 15 Number 1: Page 28.
15. Liszkowska, W. and Berlowska, J. (2021).Molecules, Yeast fermentation at low temperatures: Adaptation to changing environmental conditions and formation of volatile compounds. Volume 26 Number 4: Page. 1035.
16. Liu, Q., Li, X., Sun, C., Wang, Q., Yao, H., Yang, W., Zheng, Z., Jiang, S. and Wu, X. (2019). 3 Biotech, Effects of mixed cultures of Candida tropicalis and aromatizing yeast in alcoholic fermentation on the quality of apple vinegar. Volume 9 Number 4: Page. 128.
17. Lungaho, M., Ojuederie, O.B., Karee, K.T.. Odesola, K. A.,Popoola, J.A., Onawo, L.O. and Ikhajiagbe, B.(2025). Sustainability, Cultivation value from waste: Creating novel food,feed and industrial application from bambara groundnut by products. Volume 17 Number 16: Page. 7378
18. Maltam, D., Singh, R. and Verma, R. (2016). Journal of Industrial Microbiology and Biotechnology, Metabolic constraints affecting pentose sugar fermentation in yeast: Challenges and biotechnological prospects. Volume 43 Number 8
19. Mathew, F. and Goyal, A. (2024). In Stat Pearls. Treasure Island (FL): Stat Pearls Publishing. Ethanol.

20. Ngando S, N., Yakub, A. O., Chaulagain, D., Tangoh, A. F., Nsafon, B. E. K., Owolabi, A. B., Suh, D. and Huh, J.S. (2024). Renewable Energy, The future of clean energy: Agricultural residues as a bioethanol source and its ecological impacts in Africa. Volume 237(Part A), 121612.
21. Onyema, V. O., Amadi, O. C., Moneke, A. N. and Agu, R. C. (2023). Food Chemistry Advances, A brief review: Saccharomyces cerevisiae biodiversity potential and promising cell factories for exploitation in biotechnology and industry processes – West African natural yeasts contribution. Volume 2, 100162.
22. Onyia, I. J., George-Okafor, U. O. and Nwachukwu, U. F. (2025). International Journal of Biotech Trends and Technology, Screening for potential alcohol-tolerant yeasts from indigenous substrates as alternative to Saccharomyces cerevisiae. Volume 15 Number 1: Page. 1–7.
23. Russell, A. D. (2003). Journal of Antimicrobial Chemotherapy, Similarities and differences in the responses of microorganisms to biocides.Volume52 Number 5: Page. 750–763.
24. Saito M. (1996). Journal of Soil Science Plant Nutrition. Determination of Volatile organic acids.Volume57: Page. 247-248
25. Shah, A. M., Zhang, H., Shahid, M., Ghazal, H., Shah, A. R., Niaz, M., Naz, T., Ghimire, K., Goswami, N., Shi, W., Xia, D. and Zhao, H. (2025). Animals, The vital roles of agricultural crop residues and agro-industrial by-products to support sustainable livestock productivity in subtropical regions. Volume 15 Number 8: Page. 1184.
26. Shariq, M. and Sohail, M. (2019).Journal of King Saud University Science, Application of Candida tropicalis MK-160 for the production of xylanase and ethanol. Volume 31 Number 4: Page. 1189–1194.
27. Singh, A. K., Deeba, F. and Kumar, M. (2023). Microbial Cell Factories, Development of engineered Candida tropicalis strain for efficient corncob-based xylitol-ethanol biorefinery. Volume 22: Page. 201.
28. Sopandi, T. and Wardah, A. (2017).African Journal of Microbiology Research, Improving ethanol production by co-culturing of Saccharomyces cerevisiae with Candida tropicalis from rice husk hydrolysate media. Volume 11 Niumber 3: Page. 65–74.
29. Sorour, N., Herzallah, S., Alkhalaileh, N., Mamkagh, A., El-Baz, A., Shalaby, E., Dmoor, H. and Abbas, R. (2023). Potravinarstvo Slovak Journal of Food Sciences, Biofuel production by Candida tropicalis from orange peel waste using response surface methodology. Volume 17: Page. 862–885.
30. Sun, Y. Sun, T. Zhang, H. Lü, Z. and Yu, X. Li(2016).Journal of the Institution of Brewing, Effect of added sulphur dioxide levels on the fermentation characteristics of strawberry wine. Volume 122: Page. 446-451.

31. Walker, G. M. (2011). In Yeast Biotechnology. Springer. Pioneering research on yeast: The alcoholic fermentation of Saccharomyces cerevisiae.
32. You, J., Liu, J., Wang, X. and Zhang, H. (2017). Renewable Energy, Optimization of bioethanol production from pretreated sugarcane bagasse using yeast fermentation.Volume109: Page. 223–231.
33. Zulfikar, M., Cholis, M. and Chanson, C. (2019). Earth and Environmental Science, Molecular identification and potential ethanol production of long-term the rmotolerant yeast Candida tropicalis. Volume 239:012004.