Tanzania Journal of Science 50(3): 628-638, 2024
ISSN 0856-1761, e-ISSN 2507-7961
© College of Natural and Applied Sciences, University of Dar es Salaam, 2024

Isolation and Characterization of Yeast from Sisal Boles for Bioethanol Production

Neema Msuya*, Sospeter P Andrew, Abraham K Temu
Department of Chemical and Process Engineering, College of Engineering and Technology, University of Dar es Salaam, P. O. Box 35131, Dar es Salaam, Tanzania.
**Corresponding author: nmsuya@udsm.ac.tz; nmmsuya@gmail.com*
Received 06 February 2024, Revised 17 July 2024, Accepted Aug. 30, Publ. Sept. 30 2024
https://dx.doi.org/10.4314/tjs.v50i3.17

Abstract

Sisal boles are part of the 98% sisal plant waste that is discarded. They have a high bio-diversity of microorganism species, including yeast that can be utilized for bioethanol production. The objective of the study was to isolate yeast cells from sisal boles juice for bioethanol production. Sisal boles for juice extraction were randomly collected from Ubena sisal estate, Pwani region. To preserve the targeted microorganisms cold juice pressing method was used. Pour plate method was used on Potato Dextrose Agar and four distinct colonies were formed and repeatedly sub-cultured to obtain four pure yeast isolates. The juice yield was only 510 mL per kg of sisal bole, which is below the yield for autoclaved sisal bole obtained by other researchers. The yeast identified was concluded to be *Saccharomyces cerevisiae* based on the macroscopic and microscopic morphological observations. Isolated *S. cerevisiae* is better conditioned for fermentation of sisal bole juice to various products, including bioethanol. Upon the fermentation and temperature tolerance test, all the indigenous yeast isolates could ferment glucose, sucrose, maltose and fructose and tolerate incubation temperatures up to 45ºC. Therefore, the indigenous *S. cerevisiae* isolates can reliably be conditioned for use in ethanol production using sisal wastes.

Keywords: Saccharomyces cerevisiae; bioethanol production; fermentation efficiency; Sisal boles, Yeast Isolation

Introduction

Sisal bole is a portion of the 98% of sisal biomass which is traditionally counted as waste. Sisal bole is composed of 97.94% (w/v) organic matters in terms of Volatile solids and its juice has total sugar content of up to 30% (w/v) (Msuya et al. 2018). This sugar content is higher than that in the decortication waste and can be utilized in fermentation to produce valuable products such as bioethanol (Mshandete et al. 2013). The rising demand for bioethanol for fuel pave the way for further studies on raw materials and the isolation of yeast to minimize cost of production (Duque et al. 2021). Yeast cells are unicellular microorganisms that, along with moulds and mushrooms, are important groups of *fungi* (Singh 2023). Yeasts are widely distributed in nature within wide variety of habitats (Lachance 2020, Rosa et al. 2023). They are generally found in soil as well as on plant leaves, flowers, and fruits (Thapa et al. 2015).

Yeast isolation is the process used to grow yeast cells outside of their natural surroundings to obtain yeast cell cultures that are not mixed with other microbes, hence termed as pure culture (Gautam and Shukla 2020, Yahyapour et al. 2023). The yeast plays an essential role in ethanol production by fermenting a wide range of sugars to ethanol with valuable properties in ethanol yield (>90.0% theoretical yield), ethanol tolerance (>40.0 g/L), high ethanol

productivity (>1.0 g/L/h) and ethanol concentration (up to 120 g/l) (Azhar et al. 2017, Robak and Balcerek 2020). Other properties of yeast are that they can grow in simple, inexpensive media and undiluted fermentation broth with good resistance to inhibitors hence slows down contaminants from growth condition (Azhar et al. 2017). Various types of yeast strains such as *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* which can ferment hexoses (glucose, mannose, and galactose) as well as *Scheffersomyces stipitis*, *Kluyveromyces fagilis* and *Kluyveromyces marxianus* which can ferment both hexoses and pentoses (xylose and arabinose) have been reported to be very efficient in ethanol production from simple sugars (Azhar et al. 2017, Fernandes et al. 2022). *Saccharomyces cerevisiae* is the most common yeast cells employed in ethanol production at both industry and household levels due to its high ethanol productivity, high ethanol tolerance and ability of fermenting wide range of sugars (Azhar et al. 2017, Nuanpeng et al. 2023, Sokan-Adeaga et al. 2024).

This species tolerates acidic conditions of pH 4-6, which makes its fermentation less vulnerable to infection (Wu et al. 2022) and are known for being inhibited by pH values higher than 8.0 (Corbu and Csutak 2022). It can also tolerate ethanol better than other ethanol producing yeast cells (Varize et al. 2022) and is also generally regarded as safe for human consumption (Tesfaw et al. 2014). Strains isolated from specific regions have high adaptation to their own climatic conditions and substrate, therefore their utilization in ethanol production could be more interesting (Šimonovičová et al. 2021, Jacobus et al. 2021). Thus, the purpose of this work was to isolate *Saccharomyces cerevisiae* species from Tanzanian sisal bole for improving ethanol production from this feedstock.

Materials and Methods

Sisal boles preparation

Samples of sisal boles (Hybrid 11648) which accounts for about 80% of the sisal production in Tanzania (Monja-Mio et al. 2019, Nerini et al. 2016), were randomly collected from Ubena sisal estate, Pwani region and transported to the University of Dar es Salaam (UDSM) laboratories for the study. The leaves stubs were removed using axe and pangas and then cleaned with municipal water. The cleaned sisal boles were manually reduced in size using axe, pangas, and knives into small pieces of about 3-15 mm thick (Figure 1) in order to increase the surface area for juice extraction.

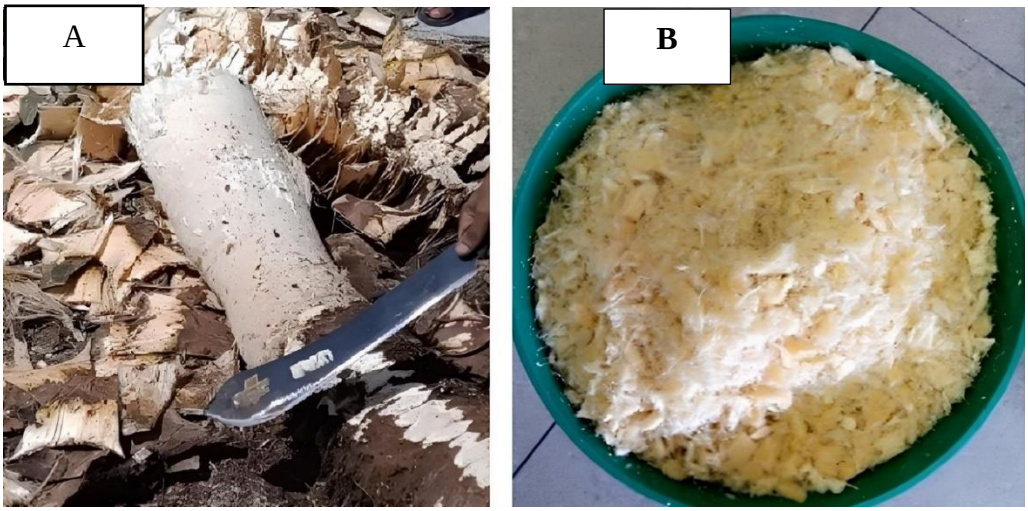

Figure 1: Sisal bole cleaning and chopping: A-Leaves stubs removal and chopping; B-Chopped sisal bole chips

Extraction of juice from sisal bole

A method by Msuya et al. (2018) was adopted for extraction of juice from sisal bole chips. This method involved direct mechanical pressing (about 7 MPa for 1 min.) of the sisal boles chips without autoclaving. Although autoclaving is preferred for higher juice yield, non-autoclaving was adopted in order to preserve the nutrients in the sisal bole juice and natural microorganisms that might have started acting on it.

Isolation and identification of yeast cells from extracted sisal bole juice

Isolation and identification of yeast cells from extracted sisal bole juice was done by adopting the method by Anyanwu et al. (2020). A sample of 0.5 ml of sisal bole juice was serially diluted at 10^{-1}, 10^{-2} and 10^{-3} using sterile distilled water. Thereafter, 0.1 ml of undiluted and diluted juice was pipetted out from the appropriate desired dilution series and poured onto separate petri plates. This was followed by pouring 20 ml of sterile Potato Dextrose Agar (YPDA -

Yeast Peptone Dextrose Adenine) medium (Fisher Scientific, Belgium) onto each plate. YPDA was used because of its suitability for the growth and flourishing of various yeast species from different environments, and is readily available in laboratories (Syrchin et al. 2023, Yuliana and Permana 2022). It is made up with potatoes which contains several nutrients essential for fungal growth and with high concentrations of amino acids that normally supports growth of auxotrophic yeast strains (Westphal et al. 2021). The plates were then gently swirled on a bench top to mix the culture and the medium thoroughly. The plates were incubated at 30°C for 48 hours, to allow for colony formation on the surface of the petri plates. Figure 2 shows the inoculated petri plates with diluted and undiluted sisal bole juice.

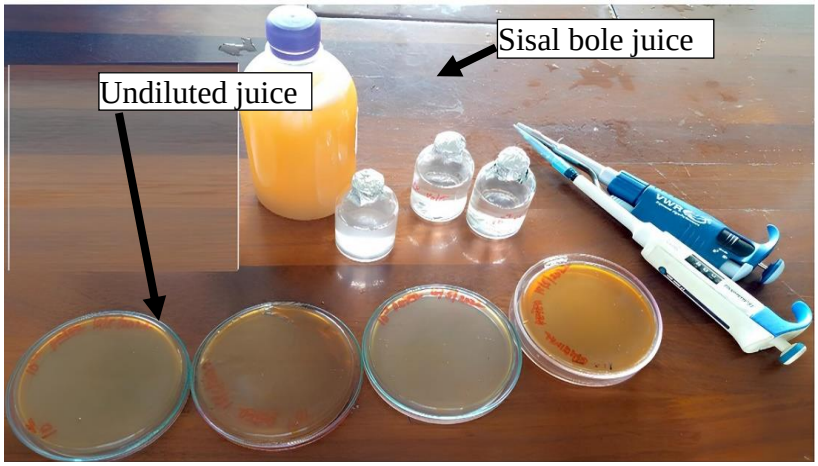

Figure 2: Inoculated petri plate with diluted and undiluted sisal bole juice

The yeast isolates or colonies formed were identified microscopically by observing the features of colony elevation, colour and other unique features. The morphology of yeast cells was detected by putting a portion of the yeast colony into a drop of lacto-phenol cotton blue on a clean glass slide. The slide was examined under the microscope using X40 objective. The identified distinct yeast colonies were sub-cultured on YPDA repeatedly isolated to obtain pure colonies. Isolation of yeast colonies was achieved using streaking methods (Kurtzman et al. 2011, Satwika et al. 2019, Mamun-Or-Rashid et al. 2022).

Carbohydrate sources for testing fermentation ability of isolated pure yeast cells

The method by Antia et al. (2018) was adopted in testing ability of the isolated pure yeast to ferment carbohydrate sources (Glucose, Fructose, Sucrose, Maltose and

Lactose) for ethanol production. Test tubes were filled with about 4-5 ml of phenol-red carbohydrate broth containing 0.5 g of peptone, 0.25 g of NaCl, 0.5 g of carbohydrate source and 20 mg of phenol red indicator in 1000 mL of distilled water. The Durham tube was inserted into the test tubes containing the broth to measure production of gas. The test media were autoclaved at 120ºC for 15 minutes for sterilization. All test tubes were inoculated with the pure yeast isolates sample using an inoculating needle loop. The test tubes were then incubated at 30ºC for 18-24 hours and observing the production of carbon dioxide gas and yellow colour formation "Positive fermentation test" (Mamun-Or-Rashid et al. 2022).

Temperature tolerance of yeast isolates

The fermentation process can be greatly affected by generated heat stress that decreases the specific growth rate of yeast strains (Yu et al. 2018). The ability of the yeast isolates to grow at higher temperatures was tested by adopting the method by Guimarães et al. (2006). Two indigenous commercial *Saccharomyces cerevisiae*, Saf-Instant (Baker's yeast) and a mutant strain (Ethanol red from France) were used as control to verify whether the strains found in this study were indigenous. The yeasts were grown on YPDA medium and incubated at different temperatures: 28, 30, 35, 37, 45 ºC and 50ºC for 24 hours. This range of temperatures was used to include the ideal temperature for yeast ethanol fermentation (30°C and 37°C), as well as temperatures lower and higher to allow for an investigation of thermotolerant ability (Azhar et al. 2017).

Results and Discussion

Extraction of juice from sisal bole

The average weight of prepared sisal bole from Ubena Sisal Estate, Pwani region was 10.4 kg. The amount of the extracted juice by direct pressing of the sisal bole chips was 510 ml per kg equivalent to 5.304 L from a single bole. The yield was slightly less than the volume reported by Msuya et al. (2018) of 567 ml of juice per kg from sisal bole collected from Fatemi Sisal Estate, Morogoro region. This could be due to the handling procedures during chips production, the age of the boles, season of harvesting or location and agronomical conditions of the collection sites. Current sisal fibre production in Tanzania is estimated to be 40,000 tonnes (Nylander 2024) and for each tonne of fibre produced 4.7 tonnes of boles are generated (Terrapon-Pfaff et al. 2012). This signifies the importance of using this waste for yeast isolation and ethanol production to reduce the environmental burden in sisal industry.

Isolation and identification of yeast cells

Macroscopic identification of yeast isolates

Colonies of yeast cells isolated from incubated sisal bole juice at 30ºC for 24 hours using Potato Dextrose Agar (PDA) medium are illustrated in Figure 3, where Y1 to Y4 are yeast strains colonies.

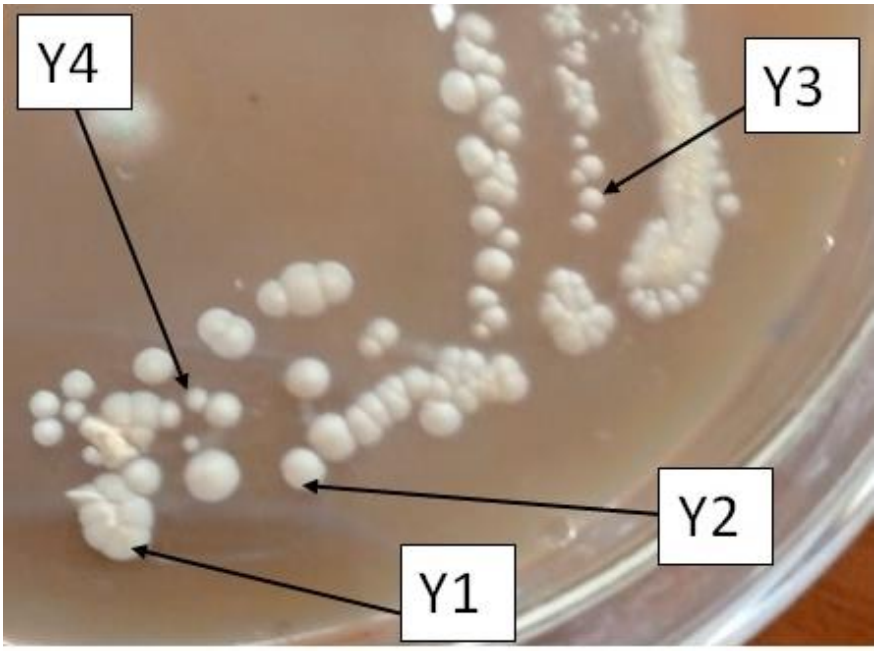

Figure 3: Colonies of yeast cells on YPDA medium as seen under microscope

Macroscopic morphological observations of the yeast isolates on the surface of the plates shows that the colonies grown on YPDA medium could be differentiated by colour (white or creamy), shape (Circular/ovoid), margin (smooth or entire), elevation (raised) and size as detailed in Table 1. Yeast cell size can vary widely in terms of length and width, depending on the species and conditions of growth. The length of cells can be small (2–6 μm), medium (5-20 μm) or large (20-50 μm) (Friedmann 2012, Walker 2011).

Table 1: Macroscopic characteristics of yeast isolates

Yeast isolates	Colour	Shape	Margin	Elevation	Size
Y1	White	Ellipsoidal to oval	Smooth	Raised	Medium
Y2	Creamy to off-white	Ellipsoidal to oval	Smooth	Raised	Medium
Y3	White	Ellipsoidal to oval	Smooth	Raised	Medium
Y4	Creamy	Ellipsoidal to oval	Smooth	Raised	Small to medium

The cellular morphology and characteristics of microorganisms clearly fit the description of saccharomyces cerevisiae yeast strain. These results are similar to the findings of Sulmiyati et al. (2019) and that of Gautam and Shukla (2020) in which all yeast isolated from different sources like kefir grain, sugarcane juice, curd, whey, and potato were identified as Saccharomyces cerevisiae strain and were morphologically characterized with white to creamy colour, round shaped, raised elevation, smooth surface, small to large in size (Table 1) and with a characteristic yeast smell.

Microscopic morphological of yeast identification

The results of microscopic morphology from yeast isolation are given in Table 2.

Table 2: Microscopic observation of yeast isolates

Yeast isolates	Cell form	Sexual reproduction	Staining colour
Y1	Oval	Budding	Blue
Y2	Spherical	Budding	Blue
Y3	Spherical	Budding	Blue
Y4	Spherical	Budding	Blue

The microscopic characteristics included oval or spherical cells with budding and blue colour of staining for all four yeast isolates as shown in Figure 4. The results reveal that, all yeast isolates are *Saccharomyces cerevisiae* species. This is in agreement with the results by Kechkar et al. (2019) who isolated yeast from sugar cane molasses.

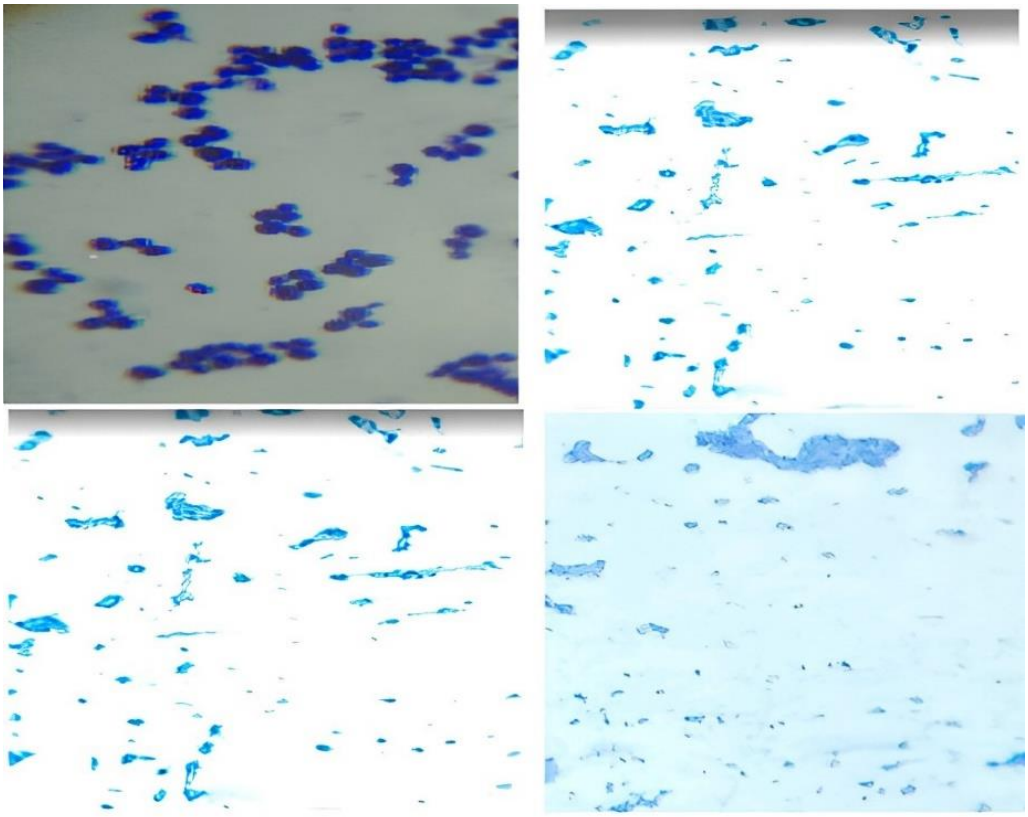

Figure 4: Microscopic observation of yeast isolates

Temperature tolerance of yeast isolates

The results of temperature tolerance for Yeast isolated and commercial *Saccharomyces Cerevisiae* are presented in Table 3. Extensive growth was seen when the ideal temperature for yeast ethanol fermentation (30°C-35°C) and below (28°C) was used. Moderate growth was observed when the temperature was 37 to 45°C but no growth was seen at a temperature of 50ºC. These results are consistent with those of Yu et al. (2018), who revealed that only yeast isolates identified as *Saccharomyces cerevisiae* were able to tolerate an incubation temperature of up to 45ºC among a large number of yeast isolates.

Table 3: Yeast isolated and commercial *S. Cerevisiae* temperature tolerance

Yeast isolates	Runs	Incubation temperature (ºC)					
		28	**30**	**35**	**37**	**45**	**50**
Y1	1	+++	+++	+++	++	++	-
	2	+++	+++	+++	++	++	-
Y2	1	+++	+++	+++	++	++	-
	2	+++	+++	+++	++	++	-
Y3	1	+++	+++	+++	++	++	-
	2	+++	+++	+++	++	++	-
Y4	1	+++	+++	+++	++	++	-
	2	+++	+++	+++	++	++	-
S. Cerevisiae	1	+++	+++	+++	++	++	-
	2	+++	+++	+++	++	++	-

Key: +++=Extensive growth, ++=Medium growth, - = No growth

Fermentation test of the yeast isolates on different carbohydrate sources

The fermentation patterns of the four yeast isolates and commercial *Saccharomyces cerevisiae* on different carbohydrates sources (Glucose, Fructose, Maltose, Sucrose and Lactose) are presented in Table 4. It was observed that, all the four yeast isolates and the commercial Saccharomyces cerevisiae used had fermentation ability.

Table 4: Fermentation Test of the Yeast Isolates on different Carbohydrate sources

Carbon source	Runs	Yeast isolates				
		Y1	Y2	Y3	Y4	*S. cerevisiae*
Glucose	1	+	+	+	+	+
	2	+	+	+	+	+
Fructose	1	+	+	+	+	+
	2	+	+	+	+	+
Sucrose	1	+	+	+	+	+
	2	+	+	+	+	+
Maltose	1	+	+	+	+	+
	2	+	+	+	+	+
Lactose	1	-	-	-	-	-
	2	-	-	-	-	-

Key: + = Positive fermentation test, - = Negative fermentation test

All the yeast strains Y1, Y2, Y3, and Y4 could ferment monosaccharides (glucose and fructose) and disaccharides (sucrose and maltose) as observed by the change of colour of phenol red carbohydrate broth from pink to yellow and the carbon dioxide gas collected in the Durham tube (Positive fermentation test). The isolated yeasts and commercial *Saccharomyces cerevisiae* could not ferment lactose since the colour of carbohydrate phenol red broth remained pink and no carbon dioxide gas was collected in the Durham tube for the duplicate experiments as shown in Figure 5(b) (Negative fermentation test). The results were similar to those reported by Yu et al. (2018) in which all *Saccharomyces* yeast isolated from different sources could not ferment lactose. Figure 5 shows the Positive and negative Durham tube Fermentation pattern of carbohydrate sources.

(a) Positive fermentation test (b) Negative fermentation test

Figure 5: Durham tube Fermentation pattern of carbohydrate sources

Conclusion

The aim of the study was to isolate yeast cells from sisal waste with focus on sisal boles that can be used for ethanol production. Pour plate method was used on Yeast Potato Dextrose Agar, whereby 0.1 mL of serially diluted at 10^{-1}, 10^{-2} and 10^{-3} sisal bole juice was inoculated and incubated at 30 ºC for 24 hours. Four distinct colonies were formed and repeatedly sub-cultured to obtain four pure yeast isolates. Macroscopic (shape, margin, elevation and size) and microscopic (cell form, sexual reproduction and staining colour) observation and morphological appearance of yeast isolated from incubation of sisal bole juice revealed that, all yeast isolates were *Saccharomyces cerevisiae* species. Preliminary assessment based on change of colour of phenol red carbohydrate and the carbon dioxide gas collected in the Durham tube (Positive fermentation test) showed that all the indigenous yeast isolates could ferment monosaccharides (glucose and fructose) and disaccharides (sucrose and maltose). Results also shows that the isolated yeast had extensive growth at the ideal ethanol fermentation temperatures and could tolerate incubation temperatures up to 45ºC. Therefore, the indigenous yeast isolates can reliably be conditioned for use in industrial ethanol production.

Acknowledgements

The authors are grateful for the financial support by Tanzania Commission for Science and Technology (COSTECH) under Bioplastics Project through University of Dar es Salaam (UDSM)-Tanzania. Authors are also grateful for technical support by Mr. Ashraf Abdi, Previous Research assistant, Bioplastics project (UDSM) and Ms. Winnie Ernest, Laboratory scientist at Microbiology Laboratory, UDSM. Special thanks to Dr. Rwaichi Minja for his constructive advice throughout data collection and manuscript preparation.

References

Antia U, Akan O, Stephen N, Eno-Ibanga C, and Akpan N 2018 Isolation and screening of yeast isolates indigenous palm wine for ethanol production. *Philippine J. Sci*. 147(3): 411-417.

Anyanwu NO, Isitua CC and Egbebi A 2020 Isolation and identification of yeast cells from palm wine in Ado Ekiti Southwest Nigeria. *Int. J. Sci. Res. (IJSR)*. 9(12): 1282-1286.

Azhar SHM, Abdulla R, Jambo SA, Marbawi H, Gansau JA, Faik AAM, and Rodrigues KF 2017 Yeasts in sustainable bioethanol production: A review. *Biochem. Biophys. Rep.*. 10: 52-61.

Corbu VM and Csutak O 2022 Molecular and physiological diversity of indigenous yeasts isolated from spontaneously fermented wine wort from Ilfov county, Romania. *Microorganisms*. 11(1): 37.

Duque A, Álvarez C, Doménech P, Manzanares P, and Moreno AD 2021 Advanced bioethanol production: from novel raw materials to integrated biorefineries. *Processes*. 9(2): 206.

Fernandes JS, de Souza ES, Carneiro ML, Silva JA, de Souza JB and Batista J 2022 Current ethanol production requirements for the yeast saccharomyces cerevisiae. *Int. J. Microb*. 1-14. doi:10.1155/2022/7878830

Friedman H (ed) 2012 Yeast: Molecular and cell biology, Second Edition, Chapter 2 - Yeast Cell Architecture and Functions pp. 5-24. Wiley Online Books. https://doi.org/10.1002/9783527659180.ch2

Gautam E and Shukla A 2020 Isolation, identification and characterization of Sacchromyces cerevisiae strains, obtained from different sources. *Int. Res. J. Eng. Techn.* 7(5): 564-575.

Guimarães TM, Moriel, DG, Machado IP, Picheth CM and Bonfim T2006 Isolation and characterization of Saccharomyces cerevisiae strains of winery interest. *Revista Brasil. Ciênc. Farmacêut.* 42: 119-126.

Jacobus AP, Gross J, Evans JH, Ceccato-Antonini SR and Gombert AK. 2021. Saccharomyces cerevisiae strains used industrially for bioethanol production. *Essays Biochem.* 65(2): 147-161.

Kechkar M, Sayed W, Cabrol A, Aziza M, Ahmed Zaid T, Amrane A and Djelal H 2019 Isolation and identification of yeast strains from sugarcane molasses, dates and figs for ethanol production under conditions simulating algal hydrolysate. *Brazil. J. Chem. Eng.* 36(1): 157-169. doi:dx.doi.org/10.1590/0104-6632.20190361s20180114.

Kurtzman CP, Fell JW, Boekhout T and Robert V 2011 Methods for isolation, phenotypic characterization and maintenance of yeasts. In CP Kurtzman, JW Fell and T Boekhout, Fifth (Ed.). *The Yeasts A Taxonomy Study*. 1: 87-110. Amsterdam: Elservier B.V. doi:10.1016/B978-0-444-52149-1.00007-0

Lachance MA 2020 Yeast selection in nature. In Yeast Strain Selection. 21-41. CRC Press.

Mamun-Or-Rashid AM, Lucy TT and Pramanik MK 2022. Isolation, identification, optimization of baker's yeast from natural sources, scale-up production using molasses as a cheap carbohydrate source, and evaluation for bread production. *Appl. Microb.* 2(3): 516-533. doi:10.3390/applmicrobiol2030040

Mshandete AM, Kibazohi O and Kivaisi AK 2013 Tanzania sisal industry: Auditing and characterization of sisal post-harvest wastes as a bio-resource for bio-refining. *Int. J. Pure Appl. Sci. Tech.* 14(2): 84.

Monja-Mio KM, Herrera-Alamillo MA, Sánchez-Teyer LF and Robert ML2019 Breeding strategies to improve production of Agave (Agave spp.). *Adv. Plant Breed. Strategies: Ind. Food Crops*.6:319-362.

Msuya N, Katima JHY, Minja RJA, Masanja, E and Temu AK 2018 Characterization of sisal boles for production of polylactic acid (PLA). *Am. J. Chem.* 8(2): 36-40.

Nerini FF, AndreoniA, Bauner D and Howells M 2016 Powering production. The case of the sisal fibre production in the Tanga region. *Tanz. Energy Policy*. 98: 544-556.

Nuanpeng S, Thanonkeo S, Klanrit P, Yamada M and Thanonkeo P 2023 Optimization conditions for ethanol production from sweet sorghum juice by thermotolerant yeast Saccharomyces cerevisiae: using a statistical experimental design. *Fermentation*. 9(5): 450.

Nylander L 2024 Corporate Commitments and Human Capabilities: A Thematic

Analysis of Corporate Social Responsibility in the Tanzanian Sisal Industry using Nussbaum's Capabilities Approach. https://www.diva-portal.org/smash/record.jsf?pid=diva2%3A1840227&dswid=-2563.

Robak K and Balcerek M 2020 Current state-of-the-art in ethanol production from lignocellulosic feedstocks. *Microb. Res.*: 240: 126534.

Rosa CA, Lachance MA, Limtong S, Santos AR, Landell MF, Gombert AK and Hallwachs W 2023. Yeasts from tropical forests: Biodiversity, ecological interactions, and as sources of bioinnovation. *Yeast*, 40(11): 511-539.

Satwika D, Djojoatmodjo S, Finnegan G, Puttajaya, D & and Ivana J 2019December. Isolation and characterization of fermenting yeast from traditional ethanol production. *Int. J. Phys: Conf. Series*. 1397(1): p. 012046. IOP Publishing. doi:10.1088/1742-6596/1397/1/012046.

Singh Y 2023 A Textbook of Fungi, Bacteria and Viruses. Academic Guru Publishing House.

Sokan-Adeaga AA, REE Ana G, Olorunnisola AO, Sokan-Adeaga MA, Roy H, Reza MS and Islam MS 2024. Ethanol production from cassava peels using Saccharomyces cerevisiae via ethanologenic fermentation process. A*rab Gulf J. Sci. Res.* 1-21

Šimonovičová A, Vojtková H, Nosalj S, Piecková E, Švehláková H, Kraková L, Drahovská H, Stalmachová B, Kučová K andPangallo D 2021. Aspergillus niger environmental isolates and their specific diversity through metabolite profiling. *Front. Microb.* 12,:658010.

Sulmiyati S, Said NS, Fahrodi D. U, Malaka R and Maruddin F 2019. The characteristics yeast isolated from commercial Kefir Grain, Indonesia. *Hasanuddin J. Anim. Sci. (HAJAS)*,.1(1): 26-36.

Syrchin SO, Yurieva OM., Pavlychenko AK, and Kurchenko IM 2023 Statistics-based optimization of cellulase and xylanase production by the endophytic fungus talaromyces funiculosus using agricultural waste materials. *Mikrobiolohichnyi Zhurnal*. 85(1): 12-25. https://doi.org/10.15407/microbiolj85.01.012

Terrapon-Pfaff JC, Fischedick M and Monheim H 2012 Energy potentials and sustainability—the case of sisal residues in *Tanzania*. *Energy Sustain. Dev.* 16(3): 312-319.

Tesfaw A, and Assefa F 2014 Current Trends in Bioethanol Production by Saccharomyces cerevisiae: Substrate, inhibitor reduction, growth variables, coculture, and immobilization. *Int. Scholarly Res. Notices*. 1-12. doi:http://dx.doi.org/10.1155/2014/532852

Thapa S, Shrestha R, Tibrewal A, Sharma A and Yuvraj KC 2015 Isolation of yeast from soil and different food samples and its characterization based on fermentation. *Nepal J. Biotech*. 3(1): 29-34.

Varize CS, Bücker A,Lopes LD, Christofoleti-Furlan,RM, Raposo,MS., Basso, LC and Stambuk BU 2022 Increasing ethanol tolerance and ethanol production in an industrial fuel ethanol Saccharomyces cerevisiae strain. *Fermentation*. 8(10): 470.

Walker G M 2011 Yeasts. In M Schaechter (Ed.), *Eukaryot. Microb.* (1st ed., pp. 3-17). Academic Press/Elsevier.

Westphal KR, Heidelbach S., Zeuner EJ, Riisgaard-Jensen M, Nielsen ME, Vestergaard SZ, Bekker NS, Skovmark J and Sondergaard TE 2021 The effects of different potato dextrose agar media on secondary metabolite production in Fusarium. *Int. J. Food Microb.* 347: 109171.

Yahyapour G, Anvar S, Ataee M, Hamed H andAskari H 2023 Isolation, Identification, and characterization of the native yeast strains from homemade cheese to assess their eliminating impact on the aflatoxin B1 and M1 of the simulated gastrointestinal fluid. *Iran J. Biotech.*. 21(2): 95-104. doi:10.30498/ijb.2023.330834.3291

Yu SZ, Htet NNW, Hlaing TS and Yu SS 2018 Isolation and characterization of effective yeast strains for bio-ethanol production. *Int. J. Plant Biol. Res*. 6(4): 1094.

Yuliana E and Permana AH 2022 Isolation and characterization of microorganisms from scoby (symbiotic culture of bacteria and yeast) during kombucha fermentation. *WARTA AKAB*, *46*(2): 18-22. https://doi.org/10.55075/wa.v46i2.140

Wu RA, Yuk HG, Liu D and Ding T 2022 Recent advances in understanding the effect of acid-adaptation on the cross-protection to food-related stress of common foodborne pathogens. *Critic. Rev. Food Sci. Nutr*. 62(26): 7336-7353.