Crossref
IJBSM October 2024, 15(10): 01-10
Article AR5597a
Research Article
Natural Resource Management
DOI: HTTPS://DOI.ORG/10.23910/1.2024.5597a

Selection and Evaluation of Thermo-tolerant Yeast Isolates for Sugar Tolerance

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ABSTRACT

The experiment was carried out during October, 2020 to January, 2022 in the Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India to evaluate the ability of thermotolerant yeast strains isolated from various fruit wastes to produce ethanol. Yeast strains should be able to grow in media with a high level of sugar concentration in order to produce high levels of ethanol. An improved yeast strain that can withstand high sugar concentrations will be able to use more carbohydrates and yield more ethanol. The ability of thermotolerant yeast strains isolated from various fruit wastes to produce ethanol was studied. A total of 100 and 10 yeasts isolates were obtained from several fruit wastes and tested for thermotolerance by growing in Yeast extract dextrose peptone broth at varied temperatures, namely 35, 40, and 45°C. Among these, 20 yeast isolates were successfully grown at 35, 40, and 45°C and were identified using morphological and biochemical methods. Further all the 20 thermotolerant isolates were screened for sugar tolerance. The broth medium was supplemented with different glucose concentrations ranging from 10 to 25% (w/v) and incubated at temperature (35, 40 and 45°C), time (24, 48, 72, and 96 h) and growth was measured by OD at 600 nm. Among 20 isolates, four (YP11, YM17, YPA48 and YPA64) were tolerant to glucose at 25.0% (w/v). The objective of this research was to isolate and characterize thermotolerant and sugar tolerant yeast to optimize maximal ethanol production from fruit waste.

KEYWORDS: Fruit wastes, optical density, sugar tolerant, thermotolerant yeast

Citation* (VANCOUVER)*: Jayamma et al., Selection and Evaluation of Thermo-tolerant Yeast Isolates for Sugar Tolerance. *International Journal of Bio-resource and Stress Management,* 2024; 15(10), 01-10. HTTPS://DOI.ORG/10.23910/1.2024.5597a.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 09th July 2024 RECEIVED in revised form on 18th September 2024 ACCEPTED in final form on 13th October 2024 PUBLISHED on 26th October 2024

1. INTRODUCTION

World demand for energy is continuing to raise significantly as a result of the rapid growth in the human population, the expansion of agriculture and industry, and the growth of the transportation industry (Gherbi et al., 2023). Increasing crude oil prices and releasing carbon dioxide and other greenhouse gases into the atmosphere from fossil fuels made alternative biofuels more attractive (Nuanpeng et al., 2023). Biofuels, such as bioethanol, biodiesel, and biogas are major renewable alternative energy sources (Hawaz et al., 2022).

Bioethanol (C_2H_5OH) is frequently produced through the bioconversion of fermentable carbohydrates using *Saccharomyces cerevisiae* (Kosaric and Velikonja 1995). *S. cerevisiae* can grow in concentrated fermentation broth, and tolerate different sugar, pH and C_2H_5OH concentrations, which resistant to inhibitors and elevated osmotic pressure, and has ethanologenic properties. (Dien et al., 2003; Olsson and Hagerdal, 1993). Nevertheless, finding the perfect yeast with strong fermentation properties-one that can endure stress factors, has outstanding invertase activity, and produces C_2H_5OH efficiently-is crucial and ongoing research (Jimenez and Benitez, 1986). The fermentation performance of yeasts is influenced by elevated temperatures encountered during ethanol fermentation processes (Meekin et al., 2002; Phisalaphong et al., 2006).

The effect of excess heat and ethanol concentration can be mitigated by using thermotolerant and ethanol-tolerant yeasts, which are compatible with simultaneous saccharifcation and fermentation at high temperatures (greater than 35°C), reducing the energy cost for cooling and the risk of contamination (Kruasuwan et al., 2023). Moreover, the increased fermentation rates at higher temperatures make the process more economical (Arora et al., 2015; Abdel-Banat et al., 2010). Several reports have described thermotolerant yeast such as *Kluyvero mycesmarxianus*, *Pichia kudriavzevii*, and *S. cerevisiae* for use in ethanol production (Limtong et al., 2007; Yuangsaard et al., 2013; Pattanakittivorakul et al., 2019; Leonel et al., 2021). Fermentation occurs between 28 and 35°C, and the maintenance of this temperature during the summer is only possible through water cooler use in fermentation tanks. Although this cooling process is crucial to maintain the temperature within an ideal range not exceeding 35°C, it is costly and requires large quantities of water (Prado et al., 2020).

Yeast should be tolerant of high concentrations of both sugar and ethanol in order for fermentations to effectively produce ethanol (Jayamma et al., 2023). The thermotolerant organisms could promote highyield of bioethanol at high temperature (Talukder, 2016). The other potential benefits of using thermotolerant yeast strains are reducing the chance of microbial contaminations and allowing long-term maintenance of anaerobic conditions, as with increasing temperature, the solubility of gases are decreased (Taylor et al., 2009; Auesukaree et al., 2012).

Tolerance of yeast to its substrate (osmotolerance), fermentation product (C_2H_5OH tolerance) and temperature (thermotolerance) has tremendous potential for application in large-scale industrial fermentation processes. Consequently, the main goals of this research were to separate and characterize yeast from various fruits and test the yeast isolate's sugar tolerance for bioethanol synthesis in various climatic conditions (Negera, 2017).

Yeast species are widely distributed in nature with divergent ecosystems and many researchers have been trying to isolate these organisms from different ecological niches such as soil in sugar cane, cassava and pineapple plantations (Kaewkrajay et al., 2014; Pongcharoen and Kawano-Kawada, 2018), rotten fruit (Chamnipa et al., 2017) or naturally fermented sources (Talukder et al., 2016) as well as clay soil (Hesham and Mohamed, 2011).

Keeping in views, the aim was to isolate and identify yeast strains from various fruit waste sources that excel in ethanol production, demonstrating thermotolerance and sugar tolerance.

2. MATERIALS AND METHODS

The investigation was conducted during October, 2020 to January, 2022 in the Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India.

2.1. Collection of samples

A total of 96 samples of various fruit wastes, including sapota (11), papaya (20), mango (16), pineapple (22), banana (10), grapes (10) and orange (07) were obtained from the various fruit markets and juice centers at Dharwad, Karnataka.

2.2. Isolation of thermotolerant yeasts

The samples were kept at 37°C for three days for enrichment of thermotolerant yeasts. From different fruit wastes, such as sapota, papaya, mango, pineapple, banana, grapes, and oranges, the isolation was done using various wastes such as sapota, pineapple, banana, grapes, papaya, mango, and oranges to obtain thermotolerant yeast strains. 10 g of fruit scraps from each waste were reduced into small fragmentsby a mortar before being added to 250 ml Erlenmeyer flasks containing 100 ml of Yeast extract peptone dextrose (YPD) broth medium, which contains 1% yeast extract, 2% peptone, and 2% glucose. The flasks were then incubated at 37°C for 72 h with periodic agitating. On YPD agar plates, isolated colonies were obtained using the spread plate technique. To further purify the colony, a single colony of the morphologically important isolate was chosen, streaked on YPD agar plates, and cultured at 37°C for 24–48 hrs. They were inoculated into YPD broth and incubated at 35, 40, and 45°C for 48–72 hrs to find thermotolerant yeast isolates. The temperature-tolerant isolates from the appropriate broth media were then streaked on YPD agar and incubated to produce pure cultures (Keo et al., 2016).

2.3. Screening of thermotolerant yeast

The thermotolerant yeast isolates were screened individually for sugar tolerance in the YPD medium. 10 g l^{-1} of medium contained yeast extract, 20 g l^{-1} of peptone and glucose added in concentrations of 10, 15, 20 and 25% (W/V) separately. The medium was sterilized and inoculated separately with each of selected yeast isolates containing 1×10^6 CFU ml^{-1}. The samples were incubated in orbital shaker cum incubator at 150 rpm, at various temperatures 35, 40, 45°C for 96 h. The initial optical density of each was taken on a spectrophotometer at 600 nm against the uninoculated medium as blank. The increase in optical density in the broth was recorded as evidence of growth (Fakruddin et al., 2013).

3. RESULTS AND DISCUSSION

By using yeast extract peptone dextrose (YPD) broth, a total of 110 yeast isolates were isolated from various fruit-wastes and tested for thermotolerance by growing at various temperatures of 35, 40, and 45°C. A total of 70 isolates survived and continued to grow at a temperature of 40°C, 20 isolates could grow at 45°C, and the greatest temperature that 30 isolates could tolerate was 35°C. It was observed that the 20 isolates grew successfully at all temperatures mentioned above. Each of the 20 thermotolerant isolate colonies were purified, then each was examined under a microscope to determine the colonies' budding characteristics. Further, the twenty thermotolerant isolates were examined for their ability to use nine different sugars, including glucose, fructose, mannose, lactose, sucrose, raffinose, galactose, arabinose, and xylose, along with the reference strain *Kluyveromyces marxianus* MTCC 4136. Only 4 of the 20 thermotolerant isolates, including the reference strain *Kluyveromyces marxianus* MTCC 4136 and strains YP11, YM17, YPA48, and YPA64, were able to utilise all nine sugars.

3.1. Screening of yeast isolates for sugar tolerance

The sugar tolerance by 20 yeast isolates along with reference strain *K. marxianus* MTCC 4136 was studied and furnished in Table 1 to 3. The experiment was conducted to evaluate the effect of initial added sugar concentration at 10.0, 15.0, 20.0 and 25.0% (w/v) on biomass production by these isolates.

The results showed that all the isolates of yeast showed good growth at 10.0% sugar concentration, whereas at 15.0%, highest OD (600 nm) was recorded by reference strain *K. marxianus* MTCC 4136 (1.22, 1.54, 1.56 and 1.56) followed by YPA64 (1.22, 1.53, 1.54 and 1.54), YM17(1.22, 1.53, 1.54 and 1.54), YP11 (1.22, 1.51, 1.53 and 1.52) and YPA48 (1.10, 1.31, 1.32 and 1.32) at 35°C for 24, 48, 72 and 96 h, respectively. At the concentration of 20% sugar *K. marxianus* MTCC 4136 (1.11, 1.34, 1.34 and 1.34) followed by YPA64 (1.11, 1.33, 1.34 and 1.34), YP11 (1.11, 1.23, 1.23 and 1.22), YM17 (1.10, 1.22, 1.22 and 1.21) and YPA48 (1.10, 1.21, 1.22 and 1.21) at 35°C for 24, 48, 72 and 96 h, respectively. Similarly at 25% sugar concentration also highest OD was recorded by reference strain *K. marxianus* MTCC 4136 (1.11, 1.23, 1.23 and 1.21) followed by YPA64 (1.10, 1.23, 1.23 and 1.21), YM17 (1.04, 1.16, 1.16 and 1.15) and YP11 (1.03, 1.13, 1.13 and 1.14) and least in YG102 (0.67, 0.70, 0.70 and 0.69) and YB74 (0.67, 0.69, 0.69 and 0.69) at 24, 48, 72 and 96 h, respectively at 35°C (Table 1).

Similarly, at 40°C the results showed that all the isolates of yeast showed good growth at 10.0% sugar concentration, whereas at 15.0%, maximum OD (600 nm) was recorded by reference strain *K. marxianus* MTCC 4136 (1.12, 1.43, 1.43 and 1.42) followed by YPA64 (1.12, 1.43, 1.43 and 1.42), YM17 (1.12, 1.39, 1.39 and 1.38), YP11 (1.11, 1.32, 1.32 and 1.31) and YPA48 (1.11, 1.32, 1.32 and 1.31), at 40°C for 24, 48, 72 and 96 h, respectively. At the concentration of 20% sugar *K. marxianus* MTCC 4136 (1.11, 1.14, 1.14 and 1.14) followed by YPA64 (1.11, 1.13, 1.14 and 1.14), YP11 (1.11, 1.13, 1.13 and 1.12), YM17 (1.10, 1.13, 1.13 and 1.12) and YPA48 (1.10, 1.11, 1.12 and 1.11) at 40°C for 24, 48, 72 and 96 h, respectively. Similarly at 25% sugar concentration also maximum OD was recorded by reference strain *K. marxianus* MTCC 4136 (1.10, 1.13, 1.13 and 1.13) followed by YPA64 (1.10, 1.13, 1.13 and 1.12), YM17 (1.01, 1.04, 1.06 and 1.05), YP11 (1.02, 1.03, 1.04 and 1.03), YPA48 (1.01, 1.02, 1.02 and 1.02) and least growth was recorded in YG102 (0.53, 0.59, 0.60, and 0.57) and YB74 (0.53, 0.59, 059 and 0.58) at 24,48,72 and 96 h, respectively at 40°C (Table 2).

Similarly, at 45°C the results showed that all the isolates of yeast showed good growth at 10.0% sugar concentration, whereas at 15.0%, highest OD (600 nm) was recorded by reference strain *K. marxianus* MTCC 4136 (1.12, 1.33, 1.34 and 1.33) followed by YPA64 (1.11, 1.32, 1.33 and 1.32), YM17 (1.03, 1.23, 1.23 and 1.23), YP11 (1.01, 1.22, 1.23 and 1.22) and YPA48 (1.02, 1.18, 1.19 and 1.19) at 40°C for 24, 48, 72 and 96 h, respectively. At the concentration of 20.0% sugar *K. marxianus* MTCC 4136 (1.11, 1.11, 1.12 and 1.12) followed by YPA64 (1.10, 1.11, 1.12 and 1.12), YM17 (1.01, 1.02, 1.02 and 1.02), YP11 (1.01, 1.01, 1.02 and 1.01) and YPA48 (1.00, 1.01, 1.02 and 1.01) at 40°C for 24, 48, 72 and 96 h, respectively. Similarly at 25% sugar

Table 1: Growth of thermotolerant yeast isolates at different concentration of glucose in yeast extract peptone broth (YEP) at 35°C

Sl. No.	Yeast isolates	Glucose concentration (% w/v) (OD values at 600 nm)											
		Control (2%)				10%				15%			
		24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h
1.	YS6	1.21	1.13	1.14	1.13	1.03	1.06	1.08	1.04	0.99	1.01	1.00	0.99
2.	YS8	1.06	1.22	1.23	1.12	1.10	1.30	1.30	1.30	0.99	1.10	1.00	1.00
3.	YP11	1.23	1.59	1.59	1.58	1.22	1.51	1.52	1.52	1.22	1.51	1.53	1.52
4.	YM17	1.22	1.52	1.53	1.52	1.20	1.51	1.52	1.52	1.22	1.53	1.54	1.54
5.	YM27	1.11	1.13	1.13	1.12	1.11	1.12	1.13	1.11	1.11	1.12	1.12	1.12
6.	YPA37	1.02	1.21	1.21	1.02	1.01	1.13	1.14	1.00	1.12	1.13	1.12	1.12
7.	YPA48	1.10	1.42	1.43	1.43	1.11	1.42	1.42	1.42	1.02	1.31	1.32	1.32
8.	YPA59	1.12	1.24	1.25	1.12	1.13	1.21	1.23	1.13	1.12	1.21	1.21	1.21
9.	YPA64	1.23	1.68	1.69	1.69	1.22	1.63	1.64	1.63	1.22	1.53	1.54	1.54
10.	YPA70	1.11	1.22	1.23	1.12	1.19	1.22	1.22	1.18	1.11	1.15	1.16	1.15
11.	YB74	1.13	1.32	1.32	1.13	1.03	1.23	1.24	1.02	1.02	1.03	1.03	1.03
12.	YB76	1.11	1.22	1.22	1.21	1.13	1.22	1.23	1.13	1.01	1.22	1.23	1.22
13.	YB85	1.12	1.45	1.45	1.43	1.15	1.21	1.22	1.15	1.02	1.11	1.12	1.12
14.	YG90	1.00	1.01	1.02	1.02	1.12	1.22	1.23	1.12	1.03	1.12	1.12	1.12
15.	YG92	1.01	1.11	1.12	1.12	1.11	1.12	1.13	1.02	1.02	1.11	1.12	1.12
16.	YG95	1.02	1.12	1.12	1.12	1.02	1.04	1.06	1.01	1.00	1.02	1.02	1.01
17.	YG100	1.00	1.10	1.11	1.11	1.06	1.07	1.08	1.05	0.99	1.00	1.00	1.00
18.	YG102	1.08	1.11	1.11	1.11	1.01	1.05	1.06	1.05	1.00	1.01	1.02	1.00
19.	YO108	1.12	1.31	1.32	1.12	1.28	1.29	1.29	1.18	1.02	1.12	1.13	1.12
20.	YO110	1.13	1.15	1.15	1.13	1.22	1.23	1.23	1.13	1.01	1.02	1.12	1.02
21.	*K. marxianus* MTCC4136	1.23	1.69	1.70	1.69	1.22	1.63	1.64	1.64	1.22	1.54	1.56	1.56

Table 1: Continue...

Sl. No.	Yeast isolates	Glucose concentration (% w/v) (OD values at 600 nm)							
		20%				25%			
		24h	48h	72h	96h	24h	48h	72h	96h
1.	YS6	0.88	0.95	0.95	0.95	0.89	0.92	0.92	0.92
2.	YS8	0.78	0.95	0.96	0.95	0.76	0.89	0.89	0.89
3.	YP11	1.11	1.23	1.23	1.22	1.03	1.13	1.14	1.13
4.	YM17	1.10	1.22	1.22	1.21	1.04	1.16	1.16	1.15
5.	YM27	0.97	1.00	1.00	0.99	0.86	0.90	0.91	0.85
6.	YPA37	0.90	1.00	1.00	0.99	0.89	0.91	0.92	0.89
7.	YPA48	1.10	1.21	1.22	1.21	1.01	1.12	1.12	1.12
8.	YPA59	0.74	0.77	0.77	0.76	0.75	0.89	0.80	0.84
9.	YPA64	1.11	1.33	1.34	1.34	1.10	1.23	1.23	1.21
10.	YPA70	0.85	0.89	0.90	0.89	0.74	0.77	0.78	0.77
11.	YB74	0.70	0.73	0.73	0.73	0.67	0.69	0.69	0.69

Table 1: Continue...

Sl. No.	Yeast isolates	Glucose concentration (% w/v) (OD values at 600 nm)							
		20%				25%			
		24h	48h	72h	96h	24h	48h	72h	96h
12.	YB76	1.00	1.11	1.12	1.11	0.99	1.00	1.00	0.98
13.	YB85	1.00	1.11	1.11	1.11	0.93	0.97	0.97	0.89
14.	YG90	0.96	1.01	1.01	1.01	0.83	0.90	0.90	0.84
15.	YG92	0.74	0.90	0.90	0.90	0.75	0.87	0.88	0.88
16.	YG95	0.75	0.97	0.98	0.97	0.72	0.89	0.89	0.89
17.	YG100	0.76	0.99	0.99	0.98	0.76	0.79	0.79	0.79
18.	YG102	0.71	0.74	0.75	0.75	0.67	0.70	0.70	0.69
19.	YO108	0.98	1.01	1.02	1.01	0.99	1.00	1.00	0.99
20.	YO110	0.95	1.01	1.01	1.01	0.94	1.00	1.00	0.98
21.	*K. marxianus* MTCC4136	1.11	1.34	1.34	1.34	1.11	1.23	1.23	1.21

Table 2: Growth of thermotolerant yeast isolates at different concentration of glucose in yeast extract peptone broth (YEP) at 40°C

Sl. No.	Yeast isolates	Glucose concentration (% w/v) (OD values at 600 nm)											
		Control (2%)				10%				15%			
		24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h
1.	YS6	1.21	1.14	1.14	1.13	1.02	1.03	1.03	1.02	0.99	1.01	1.01	0.99
2.	YS8	1.06	1.22	1.23	1.12	1.10	1.10	1.11	1.10	0.99	1.10	1.10	1.00
3.	YP11	1.13	1.48	1.49	1.47	1.12	1.40	1.40	1.40	1.11	1.33	1.32	1.31
4.	YM17	1.12	1.42	1.43	1.42	1.11	1.30	1.31	1.39	1.12	1.39	1.39	1.38
5.	YM27	1.11	1.13	1.13	1.12	1.02	1.11	1.11	1.10	1.10	1.12	1.12	1.16
6.	YPA37	1.12	1.20	1.21	1.02	1.11	1.01	1.01	0.97	1.10	1.13	1.13	1.12
7.	YPA48	1.10	1.31	1.32	1.32	1.11	1.23	1.24	1.24	1.11	1.32	1.33	1.31
8.	YPA59	1.11	1.24	1.25	1.12	1.11	1.14	1.14	1.14	1.11	1.21	1.21	1.21
9.	YPA64	1.23	1.56	1.57	1.57	1.22	1.53	1.53	1.53	1.12	1.43	1.43	1.42
10.	YPA70	1.11	1.22	1.22	1.12	1.11	1.12	1.12	1.12	1.10	1.11	1.12	1.12
11.	YB74	1.13	1.12	1.12	1.10	1.01	1.03	1.03	1.03	1.01	1.02	1.03	1.02
12.	YB76	1.11	1.21	1.22	1.11	1.11	1.13	1.13	1.12	1.10	1.02	1.02	1.02
13.	YB85	1.12	1.23	1.25	1.22	1.10	1.11	1.12	1.12	1.11	1.11	1.11	1.10
14.	YG90	1.12	1.01	1.02	1.01	1.11	1.12	1.12	1.12	1.10	1.12	1.12	1.11
15.	YG92	1.01	1.11	1.11	1.10	1.00	1.11	1.11	1.00	1.02	1.11	1.12	1.12
16.	YG95	1.02	1.12	1.12	1.02	1.02	1.02	1.03	0.97	1.01	1.00	1.00	0.99
17.	YG100	1.00	1.20	1.20	1.19	1.01	1.06	1.06	0.98	0.98	0.98	0.98	0.96
18.	YG102	0.93	1.10	1.11	1.10	1.01	1.01	1.01	0.99	0.98	0.99	0.99	0.99
19.	YO108	1.12	1.22	1.23	1.22	1.12	1.28	1.28	1.18	1.00	1.12	1.12	1.11
20.	YO110	1.13	1.15	1.15	1.13	1.01	1.22	1.22	1.12	1.01	1.01	1.01	1.01
21.	*K. marxianus* MTCC4136	1.22	1.57	1.57	1.57	1.22	1.53	1.54	1.53	1.12	1.43	1.43	1.42

Sl. No.	Yeast isolates	Glucose concentration (% w/v) (OD values at 600 nm)							
		20%				25%			
		24h	48h	72h	96h	24h	48h	72h	96h
1.	YS6	0.88	0.92	0.92	0.92	0.88	0.90	0.91	0.90
2.	YS8	0.77	0.83	0.83	0.83	0.85	0.86	0.86	0.86
3.	YP11	1.11	1.13	1.13	1.12	1.02	1.03	1.04	1.03
4.	YM17	1.10	1.13	1.13	1.12	1.01	1.04	1.06	1.05
5.	YM27	0.96	0.99	0.99	0.98	0.86	0.90	0.90	0.82
6.	YPA37	0.90	0.92	0.92	0.91	0.89	0.90	0.91	0.88
7.	YPA48	1.10	1.11	1.12	1.11	1.01	1.02	1.02	1.02
8.	YPA59	0.73	0.76	0.76	0.72	0.64	0.75	0.75	0.74
9.	YPA64	1.11	1.13	1.14	1.14	1.10	1.13	1.13	1.12
10.	YPA70	0.81	0.88	0.88	0.88	0.72	0.82	0.83	0.82
11.	YB74	0.61	0.69	0.67	0.65	0.53	0.59	0.59	0.58
12.	YB76	0.89	0.91	0.92	0.92	0.79	0.90	0.89	0.86
13.	YB85	0.82	0.89	0.90	0.88	0.92	0.93	0.93	0.88
14.	YG90	0.92	0.99	0.99	0.91	0.82	0.82	0.83	0.80
15.	YG92	0.72	0.89	0.89	0.88	0.74	0.76	0.76	0.76
16.	YG95	0.73	0.74	0.75	0.74	0.72	0.72	0.73	0.72
17.	YG100	0.74	0.87	0.87	0.85	0.74	0.75	0.76	0.72
18.	YG102	0.60	0.68	0.70	0.68	0.53	0.59	0.60	0.57
19.	YO108	0.88	0.96	0.96	0.95	0.96	0.99	0.99	0.70
20.	YO110	0.84	0.94	0.94	0.92	0.71	0.81	0.82	0.80
21.	*K. marxianus* MTCC4136	1.11	1.14	1.14	1.14	1.10	1.13	1.13	1.13

concentration also maximum OD was recorded by reference strain *K. marxianus* MTCC 4136 (1.02, 1.03, 1.04 and 1.03) followed by YPA64 (1.01, 1.02, 1.02 and 1.02), YP11 (0.93, 0.99, 1.00 and 0.99), YM17 (0.93, 0.99, 0.99 and 0.98) and YPA48 (0.91, 0.96, 0.96 and 0.95) at 24, 48, 72 and 96 h, respectively at 45°C (Table 3).

Cream-colored smooth surface, elongated, and circular colonies were chosen and purified for examination based on colony characteristics. Out of 110 yeast isolate colonies, 20 thermotolerant isolate colonies were purified, examined under a microscope, and chosen and kept for further research based on the budding characteristic. Twenty thermotolerant isolates were chosen, and they were examined for their ability to use nine different sugars, including glucose, fructose, mannose, lactose, sucrose, raffinose, galactose, arabinose and xylose, along with reference strain *Kluyveromyces marxianus* MTCC4136. Only four of the 20 thermotolerant isolates, including the reference strain *Kluyveromyces marxianus*-MTCC4136 and strains YP11, YM17, YPA48, and YPA64, were able to utilise all 9 sugars. The remaining thermotolerant yeast isolates, however, could only use glucose, fructose, mannose and sucrose.

Yeast strains with higher sugar tolerance are preferred for the ethanol fermentation process. Highly concentrated glucose impeded growth and activity of yeast. In the present investigation it was found that, at three different temperatures, including 35, 40 and 45°C, in presence of 10.0% glucose, all the thermotolerant isolates showed good growth. In the presence of 15.0% glucose, highest growth was recorded by reference strain *K. marxianus* MTCC 4136 followed by YPA64 and lowest growth was recorded by YB74 and YG102. At 20.0% glucose, maximum growth was noticed in reference strain *K. marxianus* MTCC 4136 followed by YPA64 but, least OD was recorded in YB74 and YG102. Further in presence of 25.0% glucose, maximum growth was noticed in reference strain *K. marxianus* MTCC 4136 followed by YPA64 but, least OD was recorded in YB74 and YG102. Glucose inhibition is related principally to osmotic effects (Bajpai and Margaritis, 1987).

An essential influencing factor that directly affects the rate of alcoholic fermentation and yeast cell proliferation is the high initial sugar content. A 25% (w/v) sugar concentration

Table 3: Growth of thermotolerant yeast isolates at different concentration of glucose in yeast extract peptone broth (YEP) at 45°C

Sl. No.	Yeast isolates	Glucose concentration (% w/v) (OD values at 600 nm)											
		Control (2%)				10%				15%			
		24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h
1.	YS6	0.90	0.95	0.96	0.95	0.93	0.99	1.00	0.99	0.88	0.93	0.95	0.95
2.	YS8	0.91	0.99	1.00	0.99	0.92	1.00	1.00	1.00	0.88	0.94	0.96	0.96
3.	YP11	1.04	1.33	1.34	1.33	1.03	1.33	1.34	1.33	1.01	1.22	1.23	1.22
4.	YM17	1.08	1.38	1.38	1.38	1.35	1.36	1.36	1.36	1.03	1.23	1.23	1.23
5.	YM27	0.91	0.99	1.00	0.99	0.92	0.99	1.00	1.00	0.89	0.95	0.95	0.95
6.	YPA37	0.91	1.00	1.00	1.00	0.92	1.00	1.00	1.00	0.86	0.93	0.94	0.94
7.	YPA48	1.03	1.32	1.32	1.32	1.02	1.31	1.32	1.29	1.02	1.18	1.19	1.19
8.	YPA59	0.95	0.98	0.98	0.98	0.96	0.98	0.99	0.98	0.82	0.93	0.94	0.94
9.	YPA64	1.13	1.42	1.42	1.42	1.12	1.43	1.44	1.43	1.11	1.32	1.33	1.32
10.	YPA70	0.91	0.99	0.99	0.99	0.92	0.99	0.99	0.99	0.81	0.94	0.95	0.94
11.	YB74	0.91	0.98	0.99	0.98	0.91	1.00	1.00	0.99	0.88	0.94	0.95	0.95
12.	YB76	0.91	0.98	0.98	0.98	0.92	0.99	0.99	0.99	0.82	0.95	0.96	0.95
13.	YB85	0.91	0.98	0.99	0.98	0.91	0.99	0.99	0.99	0.85	0.93	0.93	0.93
14.	YG90	0.98	1.00	1.00	0.99	0.98	1.00	1.00	1.00	0.86	0.93	0.93	0.93
15.	YG92	0.98	0.99	0.99	0.99	0.99	1.00	1.00	1.00	0.86	0.92	0.92	0.92
16.	YG95	0.99	0.99	0.99	0.99	0.99	1.00	1.00	1.00	0.84	0.91	0.91	0.91
17.	YG100	0.99	0.99	1.00	0.99	0.99	1.00	1.00	0.99	0.86	0.91	0.91	0.91
18.	YG102	0.91	0.95	0.95	0.94	0.94	0.96	0.96	0.96	0.82	0.92	0.92	0.92
19.	YO108	0.99	0.99	1.00	0.99	0.99	1.00	1.00	0.99	0.83	0.93	0.93	0.93
20.	YO110	0.92	0.96	0.96	0.96	0.92	0.96	0.97	0.97	0.85	0.93	0.93	0.93
21.	*K. marxianus* MTCC4136	1.13	1.44	1.45	1.44	1.13	1.45	1.45	1.45	1.12	1.33	1.34	1.33

Table 3: Continue...

Sl. No.	Yeast isolates	Glucose concentration (% w/v) (OD values at 600 nm)							
		20%				25%			
		24h	48h	72h	96h	24h	48h	72h	96h
1.	YS6	0.79	0.85	0.86	0.85	0.62	0.65	0.66	0.65
2.	YS8	0.79	0.85	0.86	0.86	0.65	0.66	0.67	0.66
3.	YP11	1.01	1.01	1.02	1.01	0.93	0.99	1.00	0.99
4.	YM17	1.01	1.02	1.02	1.02	0.93	0.99	0.99	0.98
5.	YM27	0.75	0.84	0.86	0.85	0.51	0.65	0.65	0.65
6.	YPA37	0.75	0.82	0.83	0.82	0.55	0.56	0.57	0.56
7.	YPA48	1.00	1.01	1.02	1.01	0.91	0.96	0.96	0.95
8.	YPA59	0.71	0.82	0.83	0.83	0.52	0.59	0.59	0.59
9.	YPA64	1.10	1.11	1.12	1.12	1.01	1.02	1.02	1.02
10.	YPA70	0.77	0.83	0.83	0.82	0.74	0.76	0.76	0.76
11.	YB74	0.54	0.59	0.59	0.58	0.36	0.46	0.46	0.45

Table 3: Continue...

Sl. No.	Yeast isolates	Glucose concentration (% w/v) (OD values at 600 nm)							
		20%				25%			
		24h	48h	72h	96h	24h	48h	72h	96h
12.	YB76	0.78	0.84	0.85	0.84	0.68	0.72	0.72	0.72
13.	YB85	0.80	0.88	0.89	0.89	0.57	0.69	0.69	0.69
14.	YG90	0.71	0.86	0.87	0.86	0.56	0.69	0.69	0.69
15.	YG92	0.79	0.89	0.89	0.89	0.55	0.67	0.67	0.67
16.	YG95	0.78	0.90	0.90	0.90	0.64	0.71	0.72	0.71
17.	YG100	0.71	0.87	0.87	0.87	0.51	0.65	0.65	0.64
18.	YG102	0.52	0.58	0.59	0.57	0.33	0.45	0.45	0.45
19.	YO108	0.75	0.85	0.85	0.85	0.71	0.73	0.73	0.73
20.	YO110	0.79	0.87	0.87	0.87	0.64	0.68	0.68	0.68
21.	*K. marxianus* MTCC4136	1.11	1.11	1.12	1.12	1.02	1.03	1.04	1.03

is shown to be tolerated by all separated yeast strains; at this point, strains Q1, B1, P1, C1, P2, and Q2 produce the highest amounts of ethanol, with maximum values of 11.45%, 9.15%, 8.35%, 7.05%, 5.45%, and 3.80% (v/v), respectively. Furthermore, the data indicates that the yield of ethanol synthesis rises with substrate concentration and reaches its maximum at 25% (w/v) concentration (Gherbi et al., 2023).

According to Lin et al. (2012) the ethanol yield produced by *S. cerevisiae* starts to decline above a substrate concentration of 30%. Similar to this, research by Triwahyuni et al. (2015) demonstrates that high sugar concentrations (25 and 30 percent (w/v)) in the fermentation environment prevent cell proliferation and the synthesis of ethanol. Water permeates the cell's cytoplasmic membrane at a concentration of 40% (w/v), which totally stops cell development by balancing intra- and extracellular concentrations. This decline in production yield may be related to the high sugar concentration that stresses the yeasts' ability to maintain osmotic balance. In the present study, of all the yeast isolates tested, four isolates YP11, YM17, YPA48 and YPA64 were found highly sugar tolerant (25.0%).

4. CONCLUSION

Twenty thermotolerant yeasts were isolated from various fruit wastes and screened for sugar tolerance and ethanol tolerance. Among the 24 isolates, YP11, YM17, YPA48 and YPA64 were found to be tolerant to glucose at 25.0% (w/v).

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