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SCREENING OF THERMOTOLENT YEAST ISOLATES FOR ETHANOL TOLERANCE AT DIFFERENT TEMPERATURES

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ABSTRACT

An ideal microorganism used for ethanol production must have rapid fermentative potential, improved flocculating ability, appreciable sugar tolerance, enhanced ethanol tolerance and good thermotolerance. The ethanol production potential of thermotolerant yeast strains isolated from different fruit waste was investigated. A total of 110 yeasts were isolated from different fruit wastes and were screened to determine thermotolerance by growing at different temperatures *viz.* 35, 40 and 45 °C by using Yeast extract dextrose peptone broth. Among these, 20 yeast isolates grew satisfactorily at all three temperatures *viz.* 35, 40 and 45°C. Further all the 20 thermotolerant isolates were screened for ethanol tolerance at all three temperatures *viz.* 35, 40 and 45 °C. The broth medium was supplemented with different ethanol concentrations ranging from 5% to 20% (v/v) at temperature (35°C, 40 °C and 45 °C), time (24 h, 48 h, 72 h and 96 h) and growth was measured by OD at 600 nm. Among 20 isolates, four (YP11, YM17, YPA48 and YPA64) were found to be highly tolerant to ethanol 20.0 per cent (v/v) at 35 °C.

KEY WORDS : Thermotolerant yeast, Sugar tolerant, Ethanol tolerant and Fruit wastes

INTRODUCTION

Yeasts are the safest and most effective microorganisms for fermenting sugars to ethanol and traditionally have been used in industry to ferment glucose based agricultural products to ethanol. Yeast is ubiquitous in the environment, but is most frequently isolated from sugar rich samples. Some good examples include fruits wastes, berries and exudates from plants. Some yeast strains are found in association with soil and insects. In assessing a yeast strain for industrial use, specific physiological properties are required. Ethanol tolerance, sugar tolerance and invertase activities are some of the important properties for use in industrial ethanol production (Jimenez and Benetez, 1986). Successful fermentations to produce ethanol using yeast require tolerance to high concentrations of both glucose and ethanol. These cellular characteristics are important because of high gravity fermentations, which are common in the ethanol industry, give rise to high sugar concentrations, at the beginning of the process, and high ethanol concentration at the end of the fermentation yeast is an important microorganism in bioindustry and its tolerance to ethanol is one of main characteristics to decide whether it can be used as biofermentation resources (Tikka *et al.,* 2013).

An ideal microorganism used for ethanol production must have rapid fermentative potential, improved flocculating ability, appreciable sugar tolerance, enhanced ethanol tolerance and good thermotolerance. Although no microbial strain has all these desirable qualities, few yeast strains have been found to possess appreciable characteristics for ethanol production. The aim of present study was to isolate and select high ethanol producer thermotolerant, sugartolerant and ethanoltolerant

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yeasts from different fruit wastes.

MATERIALS AND METHODS

Isolation of thermotolerant yeasts from different fruit waste

The samples were kept at 37 °C for three days for enrichment of thermotolerant yeasts. The isolation was carried out to obtain thermotolerant yeast strains from different fruit wastes *viz*., sapota, papaya, mango, pineapple, banana, grapes and orange were collected. Fruit wastes of each sample weighing 10 g were cut into small pieces by using mortar and pestle and placed into 250 ml Erlenmeyer flasks containing 100 ml of Yeast extract peptone dextrose (YPD) broth medium containing 1 per cent yeast extract, 2 per cent peptone, and 2 per cent glucose and incubated at 37 °C for 3 days with intermittent shaking. The spread plate technique was used to obtain isolated colonies on YPD agar plates. A single colony of the morphologically significant isolate was selected and streaked on YPD agar plates and incubated at 37°C for 24 to 48 h for further purification of the colony. For detection of thermotolerant yeast isolates, they were inoculated YPD broth and incubated at 35, 40 and 45 °C for 48 to 72h. The temperature tolerant isolates were further streaked on YPD agar from respective broth media and incubated to obtain pure cultures (Keo-Oudone *et al*., 2016).

Screening of thermotolerant yeast isolates for ethanol tolerance

Tolerance to ethanol

YPD broth medium was used for the screening of the yeasts for ethanol tolerance. The medium was autoclaved and cooled. One ml of various concentrations of absolute ethanol was varied from 5,10, 15 and 20 per cent (v/v) and was added to YPD broth separately before inoculation. Fifty millilitre of the medium was distributed into the 250 mL conical flask and then inoculated separately with each of the selected yeast isolates containing $1x10^6$ CFU ml^{-1}. The inoculated flasks were incubated at 150 rpm at various temperatures 35, 40, 45 °C for 96 h in orbital shaker cum incubator. The initial optical density of each was taken on spectrophotometer at 600 nm against the medium as blank. The increase in optical density in the broth was recorded as evidence of growth. The concentration of alcohol at which the growth of the yeast was just inhibited was assessed as the ethanol tolerance of the yeast (Nasir *et al*., 2016).

RESULTS

A total of 110 yeast isolates were obtained from different fruit wastes and were screened to determine thermotolerance by growing at different temperatures *viz.,* 35, 40 and 45 °C by using Yeast extract peptone dextrose (YPD) broth. Among all isolates, 20 were capable of growing at 45 °C and 70 isolates survived and grew at a temperature of 40 °C, while 30 could only endure a maximum of 35 °C. Finally, the results revealed that 20 isolates grew satisfactorily at all three temperatures *viz.,* 35, 40 and 45 °C. All the 20 thermotolerant isolate colonies were purified and observed under a microscope and based on the budding character, the colonies were selected and maintained for further studies

Screening of thermotolerant yeast isolates for ethanol tolerance

Data pertaining to the growth of thermotolerant yeast isolates at different ethanol levels is presented in Tables 1 to 3. The experiment was conducted to evaluate the effect of initially added ethanol concentrations of 5.0, 10.0, 15.0 and 20 per cent (v / v) on biomass production by these strains.

The results showed that all the strains showed good growth at 5.0 per cent ethanol concentration at 35°C, whereas at 10.0 per cent, maximum OD (at 600 nm) value was recorded by *K. marxianus* MTCC 4136 (1.12, 1.28, 1.28 and 1.28) followed by YPA64 (1.12, 1.28, 1.28 and 1.28), YM17 (1.10, 1.13, 1.14 and 1.13), YP11 (1.11, 1.12, 1.13 and 1.13) and YPA48 (1.01, 1.11, 1.12 and 1.11) at 24, 48, 72 and 96 h respectively. At 15 per cent the maximum OD was recorded by *K. marxianus* MTCC 4136 (1.12, 1.12, 1.13 and 1.13) followed by YPA64 (0.99, 1.19, 1.13 and 1.12), YM17 (0.89, 1.02, 1.03 and 1.02), YP11 (0.88, 1.02, 1.02 and 1.02) and YPA48 (0.84, 1.01, 1.02 and 1.01) at 24, 48, 72 and 96h, respectively. Similarly, at 20.0 per cent also, maximum OD was recorded by *K. marxianus* MTCC 4136 (1.10, 1.13, 1.14 and 1.12) followed by YPA64 (0.98, 1.03, 1.11 and 1.11), YM17 (0.83, 0.99, 0.99 and 0.98), YP11 (0.82, 0.99, 0.99 and 0.98) and YPA48 (0.85, 0.99, 0.97 and 0.97) (Table 1).

Similarly at 40 °C the results showed that all the strains showed good growth at 5.0 per cent ethanol concentration, whereas at 10.0 per cent, highest OD (at 600 nm) value was recorded by *K. marxianus*

Table 1. Growth of thermotolerant yeast isolates at different concentrations of ethanol in Yeast peptone dextrose (YPD) broth at 35°C

Sl. No.	Yeast isolates	Ethanol concentration (% v/v) (OD at 600 nm)																			
		Control (0%)				5%				10%				15%				20%			
		24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	YS6	1.12	1.15	1.14	1.13	0.94	0.99	0.98	0.97	0.76	0.82	0.83	0.82	0.48	0.57	0.58	0.57	0.32	0.35	0.35	0.35
2	YS8	1.13	1.25	1.25	1.25	0.94	0.98	0.98	0.98	0.63	0.72	0.72	0.72	0.52	0.61	0.62	0.61	0.31	0.41	0.42	0.41
3	YP11	1.21	1.30	1.31	1.32	1.11	1.22	1.23	1.22	1.10	1.12	1.13	1.13	0.88	1.02	1.02	1.02	0.82	0.99	0.99	0.98
4	YM17	1.28	1.26	1.28	1.26	1.12	1.23	1.23	1.23	1.10	1.13	1.14	1.13	0.89	1.02	1.03	1.02	0.83	0.99	0.99	0.98
5	YM27	1.18	1.25	1.25	1.25	0.99	0.99	0.97	0.95	0.70	0.84	0.85	0.85	0.52	0.68	0.68	0.68	0.31	0.36	0.37	0.36
6	YPA37	1.12	1.23	1.23	1.23	0.92	0.97	0.98	0.97	0.71	0.86	0.86	0.86	0.46	0.70	0.70	0.70	0.32	0.48	0.48	0.48
7	ypa48	1.12	1.21	1.22	1.22	1.02	1.13	1.13	1.13	1.01	1.11	1.12	1.11	0.84	1.01	1.02	1.01	0.85	0.99	0.97	0.97
8	YPA59	1.12	1.24	1.24	1.24	0.92	0.97	0.97	0.96	0.74	0.87	0.88	0.87	0.52	0.66	0.66	0.66	0.41	0.48	0.48	0.48
9	YPA64	1.20	1.28	1.29	1.28	1.13	1.32	1.33	1.32	1.12	1.28	1.28	1.28	0.99	1.12	1.13	1.12	0.98	1.03	1.11	1.11
10	YPA70	1.13	1.23	1.23	1.23	0.92	0.97	0.98	0.97	0.74	0.82	0.82	0.82	0.51	0.62	0.63	0.62	0.30	0.36	0.37	0.36
11	YB74	1.12	1.25	1.25	1.25	0.91	0.93	0.93	0.93	0.71	0.79	0.79	0.79	0.50	0.52	0.52	0.51	0.31	0.33	0.33	0.33
12	YB76	1.11	1.22	1.22	1.22	0.92	0.97	0.97	0.97	0.74	0.85	0.85	0.85	0.42	0.56	0.57	0.56	0.35	0.39	0.39	0.39
13	YB85	1.12	1.22	1.22	1.22	0.92	0.97	0.97	0.97	0.72	0.86	0.86	0.86	0.44	0.55	0.55	0.55	0.33	0.39	0.39	0.39
14	YG90	0.99	1.02	1.02	1.02	0.92	0.99	0.99	0.98	0.74	0.82	0.83	0.82	0.43	0.56	0.56	0.56	0.34	0.35	0.36	0.35
15	YG92	0.97	1.04	1.05	1.04	0.96	0.99	0.99	0.99	0.71	0.83	0.83	0.83	0.50	0.54	0.55	0.55	0.31	0.35	0.35	0.35
16	YG95	0.97	1.10	1.10	1.10	0.98	0.99	0.99	0.99	0.75	0.82	0.83	0.82	0.51	0.60	0.60	0.59	0.32	0.37	0.37	0.37
17	YG100	0.92	1.12	1.12	0.12	0.98	0.99	1.00	0.99	0.70	0.85	0.85	0.85	0.60	0.58	0.58	0.58	0.33	0.44	0.45	0.44
18	YG102	0.92	1.02	1.02	1.02	0.92	0.97	0.97	0.97	0.71	0.77	0.77	0.77	0.48	0.51	0.51	0.51	0.30	0.33	0.35	0.35
19	YO108	1.01	1.14	1.15	1.14	0.92	1.00	0.99	0.99	0.72	0.89	0.89	0.88	0.48	0.57	0.58	0.58	0.30	0.34	0.35	0.35
20	YO110	1.02	1.31	1.32	1.31	0.93	1.00	1.00	1.00	0.71	0.89	0.90	0.90	0.50	0.66	0.67	0.67	0.30	0.46	0.46	0.45
21	*K. marxianus* MTCC4136	1.20	1.28	1.29	1.29	1.13	1.33	1.33	1.33	1.13	1.28	1.29	1.28	1.12	1.12	1.13	1.13	1.10	1.13	1.14	1.12

MTCC 4136 (1.11, 1.12, 1.12 and 1.12) followed by YPA64 (1.10, 1.12, 1.12 and 1.12), YM17 (1.11, 1.13, 1.13 and 1.13), YP11 (1.10, 1.12, 1.13 and 1.12) and YPA48 (1.01, 1.02, 1.03 and 1.02) at 24, 48, 72 and 96 h respectively (Table 5). At 15 per cent the maximum OD was recorded by *K. marxianus* MTCC 4136 (1.00, 1.10, 1.11 and 1.10) followed by YPA64 (0.99, 1.10, 1.11 and 1.10) YM17 (0.84, 0.99, 1.00 and 0.99), YP11 (0.82, 0.99, 0.99 and 0.99) and YPA48 (0.83, 0.91, 0.91 and 0.91) at 24, 48, 72 and 96h, respectively. Similarly, at 20.0 per cent also, maximum OD was recorded by *K. marxianus* MTCC 4136 (0.92, 1.03, 1.03 and 1.03) followed by YPA64 (0.92, 1.01, 1.02 and 1.02), YM17 (0.73, 0.92, 0.93 and 0.92), YP11 (0.73, 0.92, 0.92 and 0.91) and YPA48 (0.72, 0.90, 0.90 and 0.90). The least OD was recorded in YG102 (0.20, 0.26, 0.27 and 0.26) and YB74 (0.13, 0.23, 0.23 and 0.22) at 24, 48, 72 and 96 h, respectively (Table 2).

Similarly at 45 °C the results showed that all the strains showed good growth at 5.0 per cent ethanol concentration, whereas at 10.0 per cent, highest OD (at 600 nm) value was recorded by *K. marxianus* MTCC 4136 (1.04, 1.05, 1.05 and 1.05) followed by YPA64 (1.03, 1.04, 1.05 and 1.04), YM17 (1.09, 1.10, 1.11 and 1.10), (1.03, 1.07, 1.07 and 1.07) and YPA48 (0.91, 0.93, 0.93 and 0.93) at 24, 48, 72 and 96 h respectively (Table 6). At 15 per cent the maximum OD was recorded by *K. marxianus* MTCC 4136 (0.99, 0.99, 1.00 and 0.99) followed by YPA64 (0.98, 0.99, 0.10 and 0.99) at 24, 48, 72 and 96h, respectively. Similarly, at 20.0 per cent also, maximum OD was recorded by *K. marxianus* MTCC 4136 (0.82, 0.90, 0.90 and 0.89) followed by YPA64 (0.81, 0.89, 0.89 and 0.89), YM17 (0.73, 0.74, 0.75

Table 2. Growth of thermotolerant yeast isolates at different concentrations of ethanol in Yeast peptone dextrose (YPD) broth at 40°C

Sl. No.	Yeast isolates	Ethanol concentration (% v/v) (OD at 600 nm) Control (0%)				5%				10%				15%				20%			
		24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	YS6	1.10	1.12	1.12	1.11	0.91	0.93	0.94	0.93	0.66	0.70	0.71	0.70	0.30	0.45	0.46	0.45	0.21	0.32	0.32	0.32
2	YS8	1.11	1.14	1.15	1.14	0.92	0.97	0.96	0.96	0.71	0.76	0.76	0.75	0.43	0.56	0.57	0.56	0.21	0.40	0.41	0.38
3	YP11	1.11	1.18	1.19	1.18	1.11	1.21	1.22	1.21	1.10	1.12	1.13	1.12	0.82	0.99	0.99	0.99	0.73	0.92	0.92	0.91
4	YM17	1.11	1.11	1.11	1.10	1.12	1.23	1.23	1.22	1.11	1.13	1.13	1.13	0.84	0.99	1.00	0.99	0.73	0.92	0.93	0.92
5	YM27	1.10	1.11	1.11	1.11	0.90	0.99	0.99	0.99	0.51	0.66	0.67	0.66	0.41	0.47	0.48	0.47	0.30	0.32	0.32	0.32
6	YPA37	1.10	1.11	1.11	1.10	0.90	0.95	0.95	0.95	0.50	0.62	0.63	0.62	0.39	0.44	0.45	0.44	0.28	0.36	0.36	0.36
7	YPA48	1.11	1.20	1.21	1.21	1.03	1.07	1.09	1.08	1.01	1.02	1.03	1.02	0.83	0.91	0.91	0.91	0.72	0.90	0.90	0.89
8	YPA59	1.01	1.03	1.04	1.03	0.92	0.94	0.95	0.94	0.53	0.67	0.67	0.67	0.43	0.44	0.45	0.44	0.25	0.35	0.36	0.35
9	YPA64	1.13	1.27	1.28	1.28	1.12	1.25	1.25	1.24	1.10	1.12	1.12	1.12	0.99	1.10	1.11	1.10	0.92	1.01	1.02	1.02
10	YPA70	1.02	1.02	1.02	1.02	0.91	0.94	0.94	0.93	0.61	0.75	0.75	0.75	0.50	0.50	0.50	0.98	0.25	0.34	0.34	0.34
11	YB74	1.01	1.04	1.05	1.04	0.90	0.92	0.92	0.91	0.58	0.61	0.61	0.61	0.32	0.41	0.41	0.40	0.13	0.23	0.23	0.22
12	YB76	1.01	1.01	1.01	1.01	0.91	0.98	0.98	0.97	0.55	0.68	0.68	0.67	0.39	0.53	0.53	0.53	0.25	0.32	0.32	0.32
13	YB85	1.02	1.01	1.03	1.02	0.91	0.99	0.99	0.99	0.51	0.64	0.64	0.64	0.38	0.41	0.42	0.40	0.25	0.31	0.31	0.30
14	YG90	0.98	0.99	1.00	0.99	0.90	0.96	0.96	0.96	0.61	0.71	0.71	0.70	0.42	0.41	0.41	0.41	0.22	0.31	0.31	0.31
15	YG92	0.96	1.00	1.01	1.00	0.95	0.98	0.98	0.98	0.64	0.65	0.66	0.65	0.49	0.43	0.44	0.43	0.23	0.29	0.29	0.29
16	YG95	0.97	0.99	1.02	0.99	0.96	0.98	0.98	0.98	0.71	0.72	0.72	0.72	0.49	0.50	0.50	0.50	0.16	0.29	0.29	0.29
17	YG100	0.92	0.98	1.02	1.02	0.91	0.99	0.99	0.99	0.78	0.81	0.81	0.81	0.31	0.52	0.52	0.52	0.19	0.30	0.30	0.30
18	YG102	0.92	0.97	1.02	0.99	0.91	0.91	0.93	0.94	0.61	0.64	0.65	0.64	0.32	0.43	0.43	0.42	0.20	0.26	0.27	0.26
19	YO108	1.01	1.13	1.14	1.13	0.91	0.96	0.96	0.96	0.41	0.66	0.66	0.66	0.31	0.54	0.55	0.54	0.21	0.29	0.29	0.29
20	YO110	1.01	1.21	1.22	1.22	0.91	0.98	0.98	0.97	0.43	0.65	0.65	0.65	0.41	0.51	0.52	0.51	0.29	0.32	0.32	0.31
21	*K. marxianus* MTCC4136	1.13	1.27	1.28	1.27	1.12	1.27	1.27	1.26	1.11	1.12	1.12	1.12	1.00	1.10	1.11	1.10	0.92	1.03	1.03	1.03

and 0.74), YP11 (0.73, 0.73, 0.74 and 0.73) and YPA48 (0.71, 0.72, 0.73 and 0.72). The least OD was recorded in YB74 (0.12, 0.13, 0.13 and 0.12) and YG102 (0.11, 0.12, 0.12 and 0.11) at 24, 48, 72 and 96 h, respectively (Table 3).

DISCUSSION

Yeast strains with higher ethanol tolerance are preferred for the ethanol fermentation process. High concentration of ethanol inhibits the growth and activity of yeast. In the present study it was observed that, at three different temperatures *viz.,* 35, 40 and 45 °C, in presence of 5.0 per cent ethanol all the thermotolerant isolates showed good growth. In the presence of 10.0 per cent ethanol, highest growth was recorded by reference culture *K. marxianus* MTCC 4136 followed by YPA64 and lowest growth was recorded by YB74 and YG102. At 15.0 per cent ethanol, maximum growth was noticed in reference culture *K. marxianus* MTCC 4136 followed by YPA64 but, least OD was recorded in YB74 and YG102. Further in presence of 20.0 per cent ethanol, maximum growth was noticed in reference culture *K. marxianus* MTCC 4136 followed by YPA64 whereas, least OD was recorded in YB74 and YG102. The ethanol tolerance capacity of yeasts mainly depends upon its unsaturated fatty acid content and also heat shock proteins produced in yeast cells. Ethanol inhibition is also linked to denaturation and inhibition of glycolytic and fermentative enzymes and modification of cell membranes (Bajaj *et al.,* 2001). Probably, the differences in ethanol tolerance in this study could also be due to the reasons mentioned above.

Laluce *et al.* (1993) also isolated

the yeast strains which had maximum alcohol tolerance up to 9.2 per cent (v/v). Unaldi *et al.* (2002) isolated yeast strains from grapes and found that the maximum alcohol tolerance of 9.0 per cent (v/v). Whereas, Nwachukwu *et al.* (2006) reported that the ethanol tolerance of the isolated yeasts revealed a wide range of ethanol tolerance levels, between 10.0 and 20.0 per cent (v/v) ethanol. Among the six isolates, Orc 6 showed the highest ethanol tolerance (20.0%) while isolates Orc 2 and Orc 11 were tolerant to 15.0 per cent ethanol (Moneke *et al.,* 2008). Similar results were observed by Tikka *et al.* (2013) who isolated seven strains of yeasts from different fruit sources which were screened for ethanol tolerance. The results showed a range of tolerance levels between 7.0 and 12.0 per cent in all the strains. Tancharoen *et al.* (2008) reported that the indigenous yeast isolate designated as BRM 17 obtained from banana, exhibited maximum ethanol tolerance upto 14.0 per cent. Brooks (2008) found out some attributes of yeast isolates for ethanol production, the ethanol tolerance of the isolates ranged from 6.0 to 12.0 per cent (v/v) ethanol. Similarly, Birch and Walker (2000) found that an ethanol concentration above 10.0 per cent (v/v) as a critical factor for yeast during the fermentation process, as a consequence of inhibition of cell division, reducing cell viability and increasing cell death. Pongcharoen *et al.* (2021) obtained two thermotolerant yeast isolates from sugarcane field soil, exhibited high temperature and ethanol tolerance levels of up to 45 °C and 15.0 per cent (v/v), respectively. Ethanol tolerance in yeast is related to environmental factors such as high sugar concentration and high temperature and is also strain

Table 3. Growth of thermotolerant yeast isolates at different concentrations of ethanol in Yeast peptone dextrose (YPD) broth at 45°C

Sl. No.	Yeast isolates	Ethanol concentration (% v/v) (OD at 600 nm)																			
		Control (0%)				5%				10%				15%				20%			
		24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	YS6	1.01	1.10	1.10	1.10	0.87	0.93	0.94	0.93	0.61	0.72	0.73	0.73	0.42	0.49	0.50	0.50	0.20	0.31	0.31	0.30
2	YS8	1.02	1.11	1.11	1.11	0.86	0.87	0.88	0.88	0.69	0.71	0.72	0.72	0.40	0.48	0.49	0.48	0.20	0.32	0.32	0.31
3	YP11	1.11	1.12	1.12	1.12	1.10	1.10	1.12	1.12	1.03	1.07	1.07	1.07	0.82	0.86	0.86	0.86	0.73	0.73	0.74	0.73
4	YM17	1.11	1.13	1.13	1.13	1.10	1.12	1.13	1.12	1.09	1.10	1.11	1.10	0.82	0.88	0.88	0.88	0.73	0.74	0.75	0.74
5	YM27	1.00	1.01	1.01	1.00	0.89	0.92	0.92	0.92	0.65	0.75	0.76	0.75	0.49	0.50	0.50	0.50	0.20	0.34	0.34	0.33
6	YPA37	1.00	1.02	1.02	1.02	0.90	0.92	0.93	0.92	0.63	0.71	0.72	0.71	0.41	0.48	0.48	0.48	0.23	0.34	0.34	0.33
7	YPA48	1.03	1.10	1.10	1.10	1.01	1.04	1.04	1.03	0.91	0.93	0.93	0.93	0.81	0.85	0.85	0.85	0.71	0.72	0.73	0.72
8	YPA59	1.00	1.01	1.01	1.01	0.91	0.92	0.93	0.92	0.63	0.69	0.70	0.70	0.41	0.48	0.48	0.48	0.22	0.22	0.22	0.22
9	YPA64	1.03	1.11	1.11	1.11	1.11	1.11	1.10	1.10	1.03	1.04	1.05	1.04	0.98	0.99	1.00	0.99	0.81	0.89	0.89	0.89
10	YPA70	1.00	1.01	1.01	1.01	0.91	0.92	0.92	0.92	0.71	0.75	0.75	0.75	0.42	0.49	0.49	0.49	0.26	0.26	0.26	0.25
11	YB74	1.01	1.01	1.01	1.00	0.82	0.83	0.83	0.83	0.41	0.51	0.51	0.51	0.31	0.38	0.38	0.04	0.12	0.13	0.13	0.12
12	YB76	1.02	1.00	1.00	1.00	0.99	1.00	1.00	0.89	0.89	0.89	0.89	0.69	0.41	0.57	0.57	0.57	0.25	0.25	0.25	0.25
13	YB85	1.01	1.02	1.02	1.01	0.88	0.90	0.90	0.90	0.64	0.70	0.70	0.70	0.46	0.49	0.49	0.49	0.22	0.24	0.23	0.23
14	YG90	0.96	0.99	0.99	1.00	0.89	0.91	0.92	0.91	0.71	0.73	0.73	0.73	0.44	0.49	0.49	0.49	0.22	0.22	0.22	0.22
15	YG92	0.95	0.99	0.99	0.99	0.91	0.92	0.92	0.92	0.70	0.75	0.75	0.75	0.42	0.49	0.49	0.49	0.14	0.17	0.18	0.18
16	YG95	0.96	0.97	0.99	0.99	0.92	0.96	0.97	0.96	0.68	0.71	0.72	0.71	0.45	0.46	0.47	0.46	0.22	0.24	0.24	0.24
17	YG100	0.91	0.96	0.96	0.96	0.85	0.91	0.92	0.91	0.66	0.73	0.73	0.73	0.36	0.40	0.40	0.40	0.21	0.25	0.25	0.24
18	YG102	0.91	0.96	0.97	0.96	0.83	0.85	0.86	0.85	0.43	0.52	0.52	0.51	0.32	0.39	0.40	0.40	0.11	0.12	0.12	0.11
19	YO108	0.99	0.99	0.99	0.99	0.90	0.88	0.88	0.88	0.68	0.67	0.67	0.67	0.39	0.42	0.42	0.42	0.29	0.30	0.30	0.29
20	YO110	0.91	0.93	0.94	0.93	0.88	0.88	0.88	0.88	0.63	0.69	0.69	0.69	0.41	0.48	0.48	0.48	0.19	0.20	0.20	0.20
21	*K. marxianus* MTCC4136	1.03	1.11	1.12	1.11	1.11	1.11	1.12	1.11	1.04	1.05	1.05	1.05	0.99	0.99	1.00	0.99	0.82	0.90	0.90	0.89

dependent. This may be related to the unsaturated fatty acid and the fatty acyl composition of the plasma membrane. Higher concentrations of membrane unsaturated fatty acids, vitamins and proteins appear to enhance ethanol tolerance. In the present investigation, of all the yeast isolates tested, four isolates YP11, YM17, YPA48 and YPA64 were found highly ethanol tolerant (20.0%).

CONCLUSION

Now a day's ethanol production by thermotolerant yeast is in demand because of their ability to grow and produce ethanol at high temperature which reduces the cooling cost during production. Ethanol and sugar tolerant characteristics of the yeast may enhance ethanol production in high sugar medium. In this study, twenty thermotolerant yeasts were isolated from different fruit wastes and were screened for sugar tolerance and ethanol tolerance. Among 20 four (YP11, YM17, YPA48 and YPA64) isolates found to be tolerant to ethanol 20.0 percent (v/v).

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