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## Isolation and identification of thermotolerant yeast isolates from different fruit wastes

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### Abstract

The application of high-potential thermotolerant yeasts is a key factor for successful ethanol production at high temperatures. In the present study, thermotolerant yeast strains were isolated from different fruit wastes. A total of 110 yeasts were isolated by an enrichment culture technique from different fruit wastes like sapota, papaya, mango, pineapple, banana, grapes and orange. Based on the typical colony characteristic on Yeast extract dextrose agar after 48 h of incubation, smooth, shiny, creamish colonies were picked up and purified and observed under a microscope based on the budding character, the colonies were selected. The selected isolates were screened to determine thermotolerance by growing at different temperatures viz. 35, 40 and 45 °C by using YPD broth. Among these, 20 isolates were capable of growing at 45°C and 70 strains survived and grew at a temperature of 40 °C, while 30 strains could only endure a maximum of 35 °C. Finally, based on the results it was concluded that 20 yeast isolates grew satisfactorily at all three temperatures viz. 35, 40 and 45 °C.

**Keywords:** Fruit waste, isolation, characterization and thermotolerant yeast

### Introduction

Ethanol production is not economically feasible in tropical countries because of the high energy requirements for cooling systems. To reduce the operating costs and to improve ethanol production efficiency, the development of a new technology is necessary. High-temperature ethanol fermentation (HTEF) is of great interest because it has several advantages, e.g., reduced cooling costs, reduced contamination risk by undesirable mesophilic microorganisms, and increased rates of sugar to ethanol conversion, increasing ethanol productivity. Many researchers have attempted to explore and characterize effective, thermotolerant, ethanol-producing yeasts that are capable of growth and ethanol production at high temperatures. Several yeast species have been characterized and classified as thermotolerant yeasts, such as *Kluyveromyces marxianus*, *Pichia* sp., *Candida* sp., and some strains of *Saccharomyces cerevisiae*. For most *Saccharomyces cerevisiae* strains, optimum temperature ranges between 25–30 °C. Some thermotolerant mutants of haploid strains of *S. cerevisiae* have been isolated using chemical and physical methods of mutagenesis and genetically characterized (Wati *et al.*, 1996) [23]. There are several reports in the literature on thermotolerant *Kluyveromyces* yeast strains (Banat *et al.*, 1992; Banat and Marchant, 1995; Singh *et al.*, 1998) [5, 4, 21]. Few reports, in comparison, describe *Saccharomyces cerevisiae* is also capable of growth and ethanol fermentation at elevated temperatures (Banat *et al.*, 1998) [6].

The fermentation efficiency of *S. cerevisiae* at high temperatures (above 35°C) is very low due to increased fluidity in membranes to which the yeasts generally respond by changing their fatty acid composition. Increasing temperature leads to higher saturated esterified fatty acids such as palmitic and palmitoleic acids in yeast cell membrane at the expense of unsaturated acyl chains such as oleic, linoleic, and linolenic acid. This is usually associated with a decrease in the amount of membrane phospholipids to maintain optimal membrane fluidity for cellular activities which are possibly part of an adaptive response (Abdel - Fattah *et al.*, 2000) [1]. It is essential for a thermotolerant yeast for enhanced ethanol production as well as to minimize the cost of energy. Hence, the present study was conducted to isolate thermotolerant yeast strains were isolated by enrichment culture technique from different fruit wastes.

### Material and Methods

#### Isolation and selection of thermotolerant yeast strains

#### Collection of samples

In this phase of the investigation, a total number of 96 samples of different fruit wastes like.

sapota, papaya, mango, pineapple, banana, grapes and orange were collected from the fruit markets located at City Bus Terminus (CBT) and various fruit juice centers of Dharwad, Karnataka, studied for the occurrence of thermotolerant yeasts strains.

### Culture Medium

Yeast extract peptone dextrose agar and Sabourds chloramphenicol agar were used for yeast isolation (Oda and Ouchi, 1989) [18].

### Isolation of thermotolerant yeasts from different fruit waste

Isolation of thermotolerant yeast strains from different fruit wastes like sapota, papaya, mango, pineapple, banana, grapes and orange by using enrichment culture technique. Fruit wastes of each sample weighing 10 g were aseptically crushed into small pieces by using mortar and pestle and placed into 250 mL Erlenmeyer flasks containing 100 mL of 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 purified colonies on YPD agar plates. For detection of thermotolerant yeast isolates, they have 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 and incubated to obtain pure cultures (Keo-oudone *et al.*, 2016) [10].

### Maintenance of cultures

The identified thermotolerant yeast isolates used for the investigation were maintained on Yeast extract peptone dextrose (YPD) agar medium.

### Characterization and identification of selected thermotolerant yeast isolates

The morphological and biochemical characteristics of twenty selected thermotolerant, yeasts strains and one reference culture of *Kluyveromyces marxianus* MTCC 4136 procured from MTCC (microbial type culture collection), Institute of Microbial Technology (IMTECH), Chandigarh, was studied according to the procedure recommended by Lodder (1970) [14].

Pure cultures of yeast strains were characterized morphologically and biochemically. Twenty colonies of fresh cultures of thermotolerant yeast isolates were observed for texture, colour, surface, elevation and margin. Cellular morphology was determined by taking a portion of the yeast colony into a drop of lactophenol cotton blue on a clean glass slide. The slide was examined under the microscope using the X40 objective. Yeast isolates were identified by standard morphological and physiological methods (Nwachukwu *et al.* 2006) [17].

### Microscopic examination of isolates

The isolates were observed microscopically for the features such as colony elevation, colour and other unique features. For microscopy, a thin smear was prepared in accordance to (Olwonibi 2017 and Yu *et al.*, 2018) [19,24].

### Biochemical characterization of the selected thermotolerant yeast isolates upon various sugars

To know the carbon utilization efficiency of the

thermotolerant yeast isolates, the thermotolerant yeast isolates were grown on various carbon sources *viz.*, Glucose, fructose, maltose, lactose, sucrose, raffinose, galactose, arabinose and xylose. Growth was identified by observing for turbidity visually (Ribereau-Gayon *et al.*, 2007) [20].

### Results

#### Isolation and selection of thermotolerant yeast strains

In the present study, a total of 110 yeast isolates were obtained from different fruit wastes. Based on the typical colony characteristic on YPD agar after 48 h of incubation, smooth ridged, shiny, creamish colonies were picked up for further study and were screened to determine thermotolerance by growing at different temperatures *viz.* 35, 40 and 45 °C by using YPD broth. Among these, 20 isolates were capable of growing at 45°C and 70 strains survived and grew at a temperature of 40°C, while 30 strains 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 (Table 1).

#### Identification of selected thermotolerant yeast isolates

Based upon the colony characters, creamish smooth surface, oval and round colonies were picked up for further study. All the 20 colonies were purified and observed under a microscope and based on the budding character, the colonies were selected, purified and maintained for further studies (Table 2).

#### Biochemical characterization of the selected thermotolerant yeast isolates

The selected twenty thermotolerant isolates along with the reference strain *K. marxianus* MTCC 4136 were tested for the utilization of nine sugars *viz.* glucose, fructose, mannose, lactose, sucrose, raffinose, galactose, arabinose and xylose respectively. Out of the twenty thermotolerant isolates, only four isolates *viz.*, YP11, YM17, YPA48, YPA64 and reference strain *K. marxianus* MTCC 4136 were able to utilize all the nine sugars. Whereas the remaining sixteen thermotolerant yeast isolates were able to utilize four sugars namely glucose, fructose, mannose and sucrose but could not grow in lactose, raffinose, galactose, arabinose and xylose. The results revealed that out of twenty isolates, only four isolates namely YP11, YM17, YPA48 and YPA64 utilized all the nine sugars (Table 3).

### Discussion

Temperature is one of the most important environmental factor which affect the microbial activity. Thermotolerant yeasts are capable of growth and fermentation during the summer months in non-tropical countries as well as under tropical climates (Ueno *et al.*, 2001) [22]. The ability of microorganisms to adopt to different temperature has attracted considerable attention, but the mechanism underlying this phenomenon is not well understood (Arthur and Watson, 1976) [3]. To grow and ferment at high temperature, all cell constituents must be stable and functional. Thermophiles appear to encompass a range of molecular mechanisms and do not appear to be due to any single factor. The fermentation efficiency of *S. cerevisiae* at high temperatures is very low due to increased fluidity in membranes to which the yeast responds by changing its fatty acids composition (Apiradee, 2006). The industry is seeking for strains that have more

tolerance to temperature and ethanol concentration during the fermentation process, which is essential for the industry.

In the present study, thermotolerant yeast isolates were isolated by enrichment culture technique. Based upon the budding characters, typical colony characteristics on YPD agar after 48 h of incubation smooth ridged, mucoid, creamish colonies were selected for further study. A total of 110 yeast isolates were selected and screened for thermotolerance to determine thermotolerant yeast isolates by growing at different temperatures viz. 35, 40 and 45 °C by using YPD broth. Among isolates, 20 yeast isolates grew satisfactorily at all three temperatures viz. 35, 40 and 45 °C. The selected twenty thermotolerant isolates along with reference strain *K. marxianus* MTCC 4136 were tested for the utilization of nine sugars viz. glucose, fructose, mannose, lactose, sucrose, raffinose, galactose, arabinose and xylose respectively. Among the twenty thermotolerant isolates only YP11, YM17, YPA48, YPA64 isolates and reference strain *K. marxianus* MTCC 4136 were able to utilize all the nine sugars. Whereas the remaining sixteen thermotolerant yeast isolates were able to utilize four sugars namely glucose, fructose, mannose and sucrose but could not grow in lactose, raffinose, galactose, arabinose and xylose.

These results are supported by similar kind of experiments which were earlier carried out by following researchers, Limtong *et al.* (2007) [13] a thermotolerant yeast strain *Kluyveromyces marxianus* DMKU 3-1042, isolated by an enrichment technique in a sugar cane juice medium supplemented with 4.00per cent (w/v) ethanol at 35 °C. Similarly, Kumar *et al.* (2009) [12] isolated thermotolerant yeasts from soil samples collected from the dumping sites of

crushed sugarcane bagasse at a sugar mill by using enrichment culture at 50°C. Koedrith *et al.* (2008) [11] isolated a thermophilic strain, *S. cerevisiae* from the banana leaves that could grow at high temperature i.e. 41- 42 °C and best suited for ethanol production. Edgardo *et al.* (2008) [8] screened eleven *S. cerevisiae* strains could grow and ferment glucose at a temperature range of 35-45 °C. They reported that all the strains were able to grow at 35 °C and 40 °C, only two strains could grow at 42 °C, and none of the strain were able to grow at 45 °C. Charoensopharat *et al.* (2015) [7] isolated thermotolerant yeasts from soil and plant materials collected from Jerusalem artichoke (JA) plantations using the YM medium supplemented with 4.00per cent (v/v) ethanol and incubation at 35°C. Various sugar fermentation tests for the identification of yeast were performed and it was observed that *Kluyveromyces* spp. showed a positive sugar fermentation pattern for lactose, sucrose, galactose, mannitol and raffinose and negative for trehalose, maltose, melibiose and rhamnose (Nahvi and Moeini, 2004) [16]. Hossain *et al.* (2020) [9] reported all the non-pathogenic yeast isolates showed positive results for fermentation of glucose, fructose, sucrose and starch and negative for Lactose.

Thermotolerant microbes are expected to be crucial for fermentation industries in tropical countries and even in non-tropical countries in summer, because they can be used for high-temperature fermentation, being stably achieved at temperatures around 40 °C, which has several advantages including reduction of cooling cost, prevention of contamination and enhancement in enzyme reaction of hydrolysis (Murata *et al.*, 2015) [15].

**Table 1:** Details of the yeast isolates obtained from different fruit wastes

Sample	No. of samples	No. of yeast isolates Tested	Code of pure yeast isolates	Thermotolerant isolates
Sapota waste	11	10	YS1, YS2, YS3, YS4, YS5, YS6, YS7, YS8, YS9 and YS10	YS6 and YS8
Papaya waste	20	06	YP11, YP12, YP13, YP14, YP15 and YP16	YP11
Mango waste	16	18	YM17, YM18, YM19, YM20, YM21, YM22, YM23, YM24, YM25, YM26, YM27, YM28, YM29, YM30, YM31, YM32, YM33 and YM34	YM17 and YM27
Pineapple waste	22	38	Ypa35, Ypa36, Ypa37, Ypa38, Ypa39, Ypa40, Ypa41, Ypa42, Ypa43, Ypa44, Ypa45, Ypa46, Ypa47, Ypa48, Ypa49, Ypa50, Ypa51, Ypa52, Ypa53, Ypa54, Ypa55, Ypa56, Ypa57, Ypa58, Ypa59, Ypa60, Ypa61, Ypa62, Ypa63, Ypa64, Ypa65, Ypa66, Ypa67, Ypa68, Ypa69, Ypa70, Ypa71 and ypa72	YPA37, YPA48, YPA59, YPA64 and YPA70
Banana waste	10	17	YB73, YB74, YB75, YB76, YB77, YB78, YB79, YB80, YB81, YB82, YB83, YB84, YB85, YB86, YB87, YB88 and YB89	YB74, YB76 and YB85
Grape waste	10	13	YG90, YG91, YG92, YG93, YG94, YG95, YG96, YG97, YG98, YG99, YG100, YG101 and YG102	YG90, YG92, YG95, YG100 and YG102
Orange waste	07	08	YO103, YO104, YO105, YO106, YO107, YO108, YO109 and YO110	YO108 and YO110
Total	96	110		20

**Table 2:** Morphological characters of the thermotolerant yeast isolates

Sl. No.	Isolate code	Colony colour	Colony nature	Elevation	Margin	Budding
1	YS6	White	Smooth and Shiny	Raised	Entire	Terminal
2	YS8	White	Smooth and Shiny	Raised	Entire	Terminal
3	YP11	White	Smooth and Shiny	Raised	Entire	Terminal
4	YM17	White	Smooth and Shiny	Raised	Entire	Terminal
5	YM27	White	Smooth and Shiny	Raised	Entire	Terminal
6	YPA37	White	Smooth and Shiny	Raised	Entire	Terminal
7	ypa48	White	Smooth and Shiny	Raised	Entire	Terminal
8	YPA59	Whitish cream color	Smooth and Shiny	Raised	Entire	Terminal
9	YPA64	Whitish cream color	Smooth and Shiny	Raised	Entire	Terminal

10	YPA70	Whitish cream color	Smooth and Shiny	Raised	Entire	Terminal
11	YB74	White	Smooth and Shiny	Raised	Entire	Terminal
12	YB76	White	Smooth and Shiny	Raised	Entire	Terminal
13	YB85	Whitish cream color	Smooth and Shiny	Raised	Entire	Terminal
14	YG90	Whitish cream color	Smooth and Shiny	Raised	Entire	Terminal
15	YG92	Whitish cream color	Smooth and Shiny	Raised	Entire	Terminal
16	YG95	Whitish cream color	Smooth and Shiny	Raised	Entire	Terminal
17	YG100	White	Smooth and Shiny	Raised	Entire	Terminal
18	YG102	White	Smooth and Shiny	Raised	Entire	Terminal
19	YO108	White	Smooth and Shiny	Raised	Entire	Terminal
20	YO110	White	Smooth and Shiny	Raised	Entire	Terminal
21	<i>K. marxianus</i> MTCC4136	Whitish cream color	Smooth and Shiny	Raised	Entire	Terminal

**Table 3:** Utilization of sugars by the thermotolerant yeast isolates

Sl. No.	Yeast isolate	Glucose	Fructose	Mannose	Lactose	Sucrose	Raffinose	Galactose	Arabinose	Xylose
1	YS6	+	+	+	-	+	-	-	-	-
2	YS8	+	+	+	-	+	-	-	-	-
3	YP11	+	+	+	+	+	+	+	+	+
4	YM17	+	+	+	+	+	+	+	+	+
5	YM27	+	+	+	-	+	-	-	-	-
6	YPA37	+	+	+	-	+	-	-	-	-
7	ypa48	+	+	+	+	+	+	+	+	+
8	YPA59	+	+	+	-	+	-	-	-	-
9	YPA64	+	+	+	+	+	+	+	+	+
10	YPA70	+	+	+	-	+	-	-	-	-
11	YB74	+	+	+	-	+	-	-	-	-
12	YB76	+	+	+	-	+	-	-	-	-
13	YB85	+	+	+	-	+	-	-	-	-
14	YG90	+	+	+	-	+	-	-	-	-
15	YG92	+	+	+	-	+	-	-	-	-
16	YG95	+	+	+	-	+	-	-	-	-
17	YG100	+	+	+	-	+	-	-	-	-
18	YG102	+	+	+	-	+	-	-	-	-
19	YO108	+	+	+	-	+	-	-	-	-
20	YO110	+	+	+	-	+	-	-	-	-
21	<i>K. marxianus</i> MTCC4136	+	+	+	+	+	+	+	+	+

## Conclusion

Currently, majority of bioethanol is produced using mesophilic microorganisms. However, thermo-ethanogenic yeasts receive considerable interest due to the current challenges of increasing temperature, which could potentially overcome many obstacles. The use of thermophilic or thermotolerant yeast for bioethanol production has several process advantages including broad substrate utilization range, higher saccharification and fermentation rates, minimized contamination risk, lower costs of pumping and stirring and no cooling problems, less energy requirement for mixing and product recovery. With the above advantages in mind, the present investigation was carried out on the isolation and identification of thermotolerant yeast isolates from different fruit waste.

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