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Isolation Identification And Characterization Of Thermophilic Fermenting Yeast From The Jaiselmer

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Abstract

Due to a current challenge of increasing global temperature, thermo-ethanologenic yeasts receive considerably interest nowadays. In this study, 10 yeast isolates were checked for their thermophilic growth at temperature 40^{0}-45^{0}C four of them were selected for their fermentative capacity and ethanol tolerance at high temperatures. Only JCY2 was able to grow at 45^{0}C. Four isolates JCY2, JCY3, JBY1, SF112 performed significantly well (at the 95% confidence level) in fermentation at 43^{0}C. Of which JCY2was able to tolerate up to 20% v/v ethanol as compared to*S. cerevisiae*. Therefore only one isolate having high ethanol tolerance activity was selected indicating the further application feasibility of this yeast for ethanol production at high temperature was characterized.

Introduction

Ethanol production by thermotolerant yeasts have been extensively studied because thermotolerant yeasts are capable of growth and fermentation during the summer months in non-tropical countries as well as under tropical climates.Travassos and Cury (1966) defined thermophilic yeasts as those growing optimally at $\geq 37^{0}$C. The aim of this study was to isolate and characterize thermotolerant yeast to produce ethanol. Thermotolerant and or thermophilic micro-organisms are very useful for certain industrial processes (Banat *et al.*,1998; Banat & Marchant, 1995; Kadam&

Schmidt, 1997). The production of biological materials at high temperatures rather than the customary practice makes it possible to reduce the risk of contamination and the operation costs of maintaining growth temperatures in large-scale systems, and to increase the rate of productivity, etc. (Nolan *et al*., 1994). For these reasons, many efforts have been made to seek or develop thermotolerant and or thermophilic strains (Gera *et al*., 1997; KiranSree*et al*., 2000). The thermopile yeasts were reported to the heat stability of intracellular membranes. When these membranes are heat labile the yeasts are not thermophilic. An important component of membranes in temperature adaptation is unsaturated fatty acid. Psychrophilic yeasts have a high content of unsaturated fatty acids (Kocková-Kratochvílová, 1990).Thermotolerance may be defined as the transient ability of cells subjected to high temperatures to survive subsequent lethal exposures to elevated temperatures (Laszlo, 1988). Yeast cells exhibit a rapid molecular response when exposed to elevated temperature. This is called the heat-shock response and is a ubiquitous regulatory phenomenon in all living cells. Sub-lethal heat-shock treatment of yeast leads to the induction of synthesis of a specific set of proteins, the highly conserved"heat-shock proteins" (Hsps) (Walker, 1998). In general, industrial yeast strains are able to grow and efficiently fermentethanol at pH values of 3.5-6.0 and temperatures of 28-30° C, with efficiency dropping off rapidly at higher temperature. Thermotolerant yeasts were isolated from fruit (Banana), curd and roots of some xerophytic plants collected from SAM desert (Jaiselmer) were used. All isolates of yeasts were screened and selected by considering the viability and ethanol fermentation at 40^0C. The optimization of some ethanol production conditions was investigated. Selected thermotolerant yeast isolate was identified using morphological, physiological and biochemical characteristics. Genomic analysis of the selected isolated was also performed.

Materials Method

Study area, sampling and yeast isolation

Yeast cultures were isolated from fruit (Banana), curd and roots of some xerophytic plants collected from SAM desert Jaiselmer(altitude-225M; latitude $26^0.4^{'}$-N; $69^0.20^{'}$-E Rajasthan, India. Initially samples were inoculated in broth containing 2% D-glucose and 0.5% yeast extract and the tube was incubated at room temperature on rotary shaker (150 rpm) for 24 hrs. When growth (turbidity) was detected (visually), a loopful of suspension was inoculated onto PDA/YEPD/YGC.

Screening of thermotolerant yeast strains

Two strategies were adopted –

One plate incubated with source material was incubated at 45^0C for 120 hrs in a container containing thin film of water to avoid drying of medium.

The isolated yeasts were incubated on to PDA medium, and plate was incubated at 45^0C for 72 hrs as above.

Screening of thermotolerant ethanol fermenting yeasts

The efficiency of ethanol production of thermotolerant yeast strain was tested as earlier (Sreet et al., 2000 ; Ueno et al., 2001). 15 ml of YWPD (pH 5.5) broth was taken in durham tube (Durham E, 1898) and 0.1 ml of cell suspension (OD_{620} =0.1)of log phase cell was inoculated under sterile condition. The inoculated durham tube was incubated at 40^0C and 43^0C for three days. The tubes were shaken gently at 24hr interval to create partial aerobic condition Fermentation was recognized by the accumulation of CO_2 gas trapped in the inner durham tube

(Ueno et al., 2001 ; Ueno et al., 2002). The ethanol production was determined by using a gas chromatograph or byCaputi et al., (1968).

Ethanol tolerance study

Isolated yeasts were examined for their growth rate and tolerance to ethanol. Different strains were incubated in 1ml YEPD medium supplemented with different ethanol concentrations such as 10%, 13%,14%, 16%,18% and 20% (v/v) in separate microtubes. These tubes were incubated for three days at 30^0C. These yeast strains were then diluted in sterile water (OD_{620} =0.1) and 1µl of each of which was dropped on to the YEPD- agar medium (drop assay) and incubated at 30^0C for 24 hrs. The compact patch forming yeast in the inoculated area would rander it more tolerant than that forming diffuse patch.

Identification

Thermotolerant yeast was examined for ascospore formation applied from Kurtzman*et al*. (2005).Morphological characterization done by method of Kreger-van Rij (1984) and Kurtzman and Fell(1997),

Growth on solid medium

The morphology of cells of thermotolerant yeast and their appearance on solid medium, on YPD agar was examined, after incubating at 45^0 for 3 days. The following features of the appearance of cultures were recorded; texture, color and surface of colonies. Their ascospore and pseudo-mycelium formation were determined.

Taxonomic identification of Yeast strains

The colony and cell structure of JCY2 were analysed according the method given earlier (Kurtzmanet al., 2005). Molecular identification of JCY2 was performed on

the basis of sequence characteristics of D1/D2 domain and ITS and 5.8s region of the largerDNA, respectively. DNA isolation from yeast was per-formed as per the protocol given earlier (Harju 2004). Sequences were compared with the nonredundant NCBI database using BLASTN, with the default settings used tofind the most similar sequences, and were sorted by the E score. A representative sequence of 10 most similar neigh-bours was aligned using CLUSTAL W2 for multiple align-ments with the default settings. The multiple-alignment file was then used to create phylogenetic trees applying UPGMA method in MEGA5 software.

Results and discussion

Screening of thermotolerant yeasts

About nine yeasts isolates (SW1), (JBYI), (JBY2), (JSY1), (JSY2), (BHY1), (JCY2), (SF112), (JCY3) were tested for their ability to grow at higher between 40-45^{0}C. The morphology of cells of thermotolerant yeast and their appearance on solid medium, (YEPD agar) was examined, after incubating at 45°C for 3 days. It was observed that morphology of JCY2 was good (texture, color and surface of colonies). JCY2 was the only isolate that could grow at 45^{0}C (Figure 1)

Effect of temperature on fermentation

The isolates were inoculated in the yeast fermentation medium (YEM) in Durham tubes and incubated at 15^{0}C, 21^{0}C, 43^{0}C. The yeast (*S. cerevisiae)* was used as control. Table 1shows that except *S. cerevisiae*all the isolates are thermotolerants.

Ethanologenic characterization of the isolates

Isolated yeasts were examined for their growth rate and tolerance to ethanol. Different strains were incubated in 1ml YEPD medium supplemented with different ethanol concentrations. These tubes were incubated for three days at 30^0C. These yeast strains were then diluted in sterile water and spotted (drop dilution assay) on YEPD. From this experiment it was concluded that JCY2 showed more viable colony along with *S. cerevisiae* which is used as control in all concentration of ethanol. The isolate JCY2 showed more viable cells and hence more compact colony (Figure2). The effect of temperature on the growth of yeast was study under the range of 40 and 45^0C, and the result was showed in (Figure 1). At temperature higher than 40^0C, the growth of yeast was decreased. In this study, ethanol fermentation was occurred at temperature higher than optimum temperature because yeast currently used for industrial fermentation are rapidly inactivated at 33-35°C (Laluce*et al*., 1987). Significant cooling costs would be eliminated, especially during the summer or in tropical countries, with fermenting temperatures of 40^0C and above. Additional energy requirements involving heating and subsequent cooling, often required in the preparation of many substrates for fermentation, could be drastically reduced in processes operated at these temperatures. Fermentation at temperatures higher than the optimum requires the selection of unconventional yeast starters capable of eluding the inhibitory effect of ethanol, which is increasing with temperature, and consequently of 10 µm 49 attaining maximum yield levels. Only a few screening surveys have been carried out for the ability of yeasts to grow and ferment at or above 40 °C (D'Amore*et al*., 1989, Anderson *et al*., 1986 and Hacking *et al*., 1984).

Ascospore formation

The cells formed ascospore 4 ascospores per cell.

Physiological characterization

Aerobic carbon and nitrogen utilization

The ability of JCY2 vis-a-vis*C. krusei* (Telemorphic*I. orientalis*) to utilize various carbon and nitrogenous compounds as sole source of carbon and nitrogen respectively is shown in tables 2, 3, 4,5.

The tables showed that the carbon and nitrogen utilization patterns of JCY2 and type strain of *Issatchenkia orientalis or* its anamorph *Candida krusei* are almost same.

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Table 1. Fermentation of glucose by yeast isolates at different temperatures

S.NO.	Fermentation at different temperatures (^{0}C)			
	Yeast strains	15	21	43
1.	JCY2	+	+	++
2.	JCY3	+	++	++
3.	JBY1	+	++	++
4.	SF112	++	++	++
5.	*S. cerevisiae*	++	++	--

Table 2 Aerobic utilization of different carbon compounds as carbon sole source

S. No	Isolates	Aerobic Utilization of carbon sources												
		D-Glucose	D-Arabinose	L-Arabinose	L-Sorbose	Sucrose	D-Ribose	D -Xylose	Galacitole	Myo-inositole	Erthritol	L-Rhamnose	Urea	Maltose
1	JCY$_2$	+	-	W	W	-	-	W	-	-	-	+	+	+
2	*C. krusei*	+	-	W	W	-	-	-	-	-	-	+	+	+

(-) No growth (+) positive growth (w) weak growth

Table 3 Aerobic utilization of different carbon compounds as sole carbon source

S.NO	Isolates	Aerobic Utilization of carbon sources												
		Trehlose	Pectin	Cellobiose	Salicin	Melibiose	Lactose	Raffinose	Melizitose	Insulin	Starch	Glycerol	Erythritol	L-
1.	JCY2	-	W	-	W	-	-	-	-	W	-	+	-	-
2	*C krusei*	-	-	-	-	-	-	-	-	-	-	+	-	-

(-) No growth (+) positive growth (w) weak growth

Table 4 Aerobic utilization of different carbon compounds as sole carbon source

S.NO	Isolates	Aerobic Utilization of carbon sources												
		Xylitol	D-mannitol	Sorbitol	Casein	DL-Lactate	Succinate	Citrate	Methanol	Ethanol	DMethylglucosid	Propanal	ButanoL	Quinicacid	Arabinitol
	JCY2	-	-	+	W	+	+	W	-	+	+	-	-	+	-
2	*C krusei*	-	-	-	-	+	+	-	-	+	+	-	-	+	-

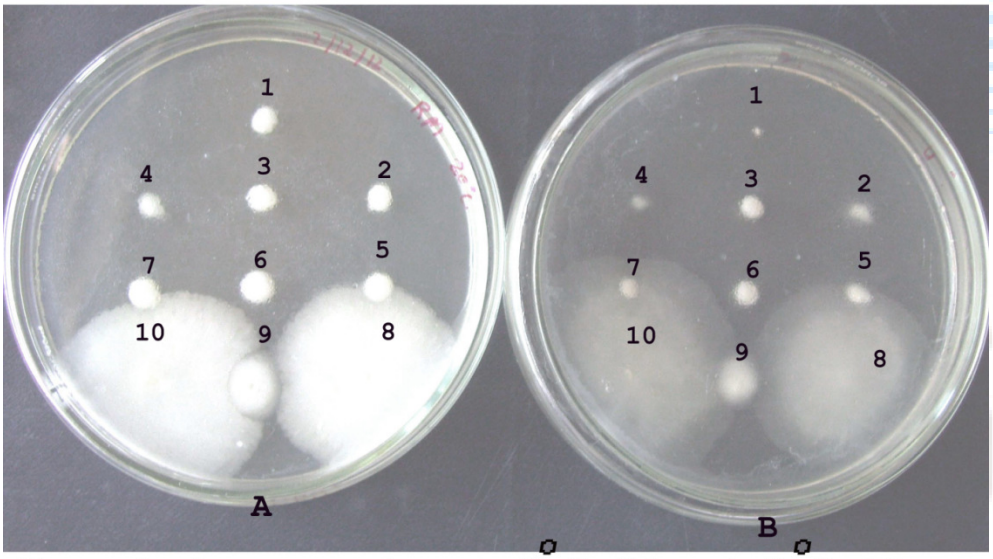
(-) No growth (+) positive growth (w) weak growth

Table 5 Aerobic utilization of different nitrogenous compounds as sole nitrogen source

S.NO.	Isolates	Aerobic Utilization of nitrogen sources										Growth	
		Nitrite	Nitrate	Ethylamine	Lysine	Cadaverine	Creatine	Creatinine	Glucosamine	Imidazol	Tryptophan	0.01% Cycloh.	0.1% Cycloh.
1	JCY2	-	+	+	W	W	+	+	-	-	+	-	-
2	*C krusei*	-	+	+	-	+	-	-	-	-	+	-	-

(-) No growth (+) positive growth (w) weak growth

.



Yeast-isolates growing at 40 C (A) and 45 C (B)

Figure 1 A&B Effect of temperature on the growth of yeast isolates; 1 (*S. cerevisiae*), 2 (SW1),3 (JBYI), 4(JBY2), 5(JSY1), 6(JSY2), 7(BHY1), 8(JCY2), 9(SF112), 10(JCY3)

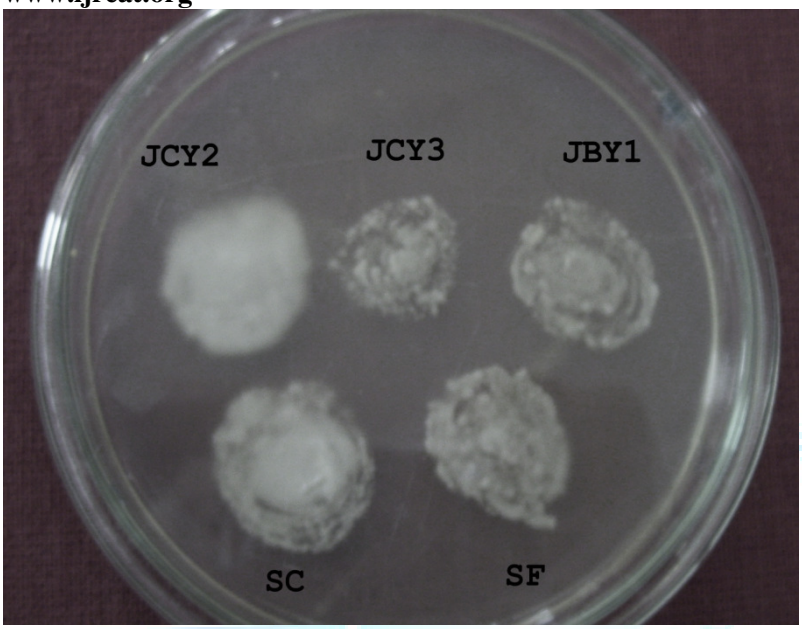


Figure 2 Ethanol tolerance (20% v/v) by yeast-isolates

Molecular characterization

>JCY2

CAAAGCGGAGGAAAAAGAAACCAAAAGGGATTGCCTCAGTAGCGGCGAGTGAAGCGGCAAGAGCTC
AGATTTGAAATCGTGCTTTGCGGCACGAGTTGTAGATTGCAGGTTGGAGTCTGTGTGGAAGGCGGTGT
CCAAGTCCCTTGGAACAGGGCGCCCAGGAGGGTGAGAGCCCCGTGGGATGCCGGCGGAAGCAGTGAG
GCCCTTCTGACGAGTCGAGTTGTTTGGGAATGCAGCTCCAAGCGGGTGGTAAATTCCATCTAAGGCTA
AATACTGGCGAGAGACCGATAGCGAACAAGTACTGTGAAGGAAAGATGAAAAGCACTTTGAAAAGAG
AGTGAAACAGCACGTGAAATTGTTGAAAGGGAAGGGTATTGCGCCCGACATGGGGATTGCGCACCGC
TGCCTCTCGTGGGCGGCGCTCTGGGCTTTCCCTGGGCCAGCATCGGTTCTTGCTGCAGGAGAAGGGGTT
CTGGAACGTGGCTCTTCGGAGTGTTATAGCCAGGGCCAGATGCTGCGTGCGGGGACCGAGGACTGCGG
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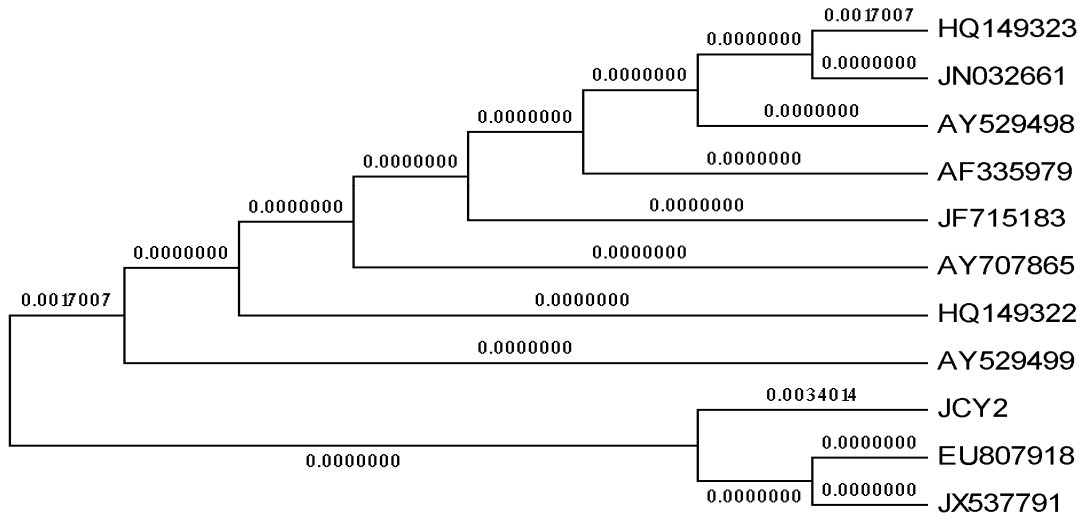

Figure 3 Sequence of ITS region of rRNA gene &Dendrogram showing phylogenetic relationship of JCY2 with 10 most closely related strains in the Gene Bank database