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RESEARCH ARTICLE

THREE NOVELS THERMOTOLERANT ETHAN0LOGENIC *Saccharomyces cerevisiae* STRAINS FOR HIGH ETHANOL PRODUCTION FROM HIGH GRAVITY SUGAR CANE MOLASSES

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ABSTRACT

There is a need for new approaches to isolate thermotolerant yeast strains that can be utilized for the efficient production of ethanol .The application of high-potential thermotolerant yeasts is a key factor for successful ethanol production at high temperatures The use of thermotolerant yeast is a vital to avoid the use of expensive cooling systems. In addition, avoids fermentation stops due to overheating, reducing the chance of infection and minimizes the volume of wastewater generated in the distilleries. Ninety strains of *Saccharomyces cerevisiae* were screened on sugar cane molasses medium for ethanol production .The higher fourteen strains were subjected to select the more osmotolerant strains on high gravity sugar cane molasses medium(20% w/v fermentable sugars), Thee promising osmotolerant were testing to select the faster fermentation rate .Three most patents were subjected to different temperature from 40°C to 55°C f0r different periods i.e 20 min to 18 hrs. The thermal tolerance of three selected strains were evaluated at temperatures between 28 to 44 °C. The high ethanol yield 11.42 % v/v, 11.55 % v/v and 11.64 at 40 °C were achieved by *S. cerevisiae*F-96, *S. cerevisiae*F-50and *S. cerevisiae* FE-403 respectively comparable to 9.65 % v/v,9.45 and 8.90 % v/v at 40°C achieved with the same strains respectively before heat shock treatment. Additionally, more than 96 % fermentable sugars utilization was achieved.

INTRODUCTION

There is a need for new approaches to isolate thermotolerant yeast strains that can be utilized for the efficient production of ethanol (West and Kennedy, 2014). The application of high-potential thermotolerant yeasts is a key factor for successful ethanol production at high temperatures (Techaparina *et al*., 2017). The use of thermotolerant yeast is a vital to avoid the use of expensive cooling systems. In addition, avoids fermentation stops due to overheating, reducing the chance of infection as well as minimizes the volume of waste water generated in the distilleries. Amongst the options available for an organization pursuing industrial microbiology to help increases its profits in the face of its "Competitors race" for the world market, strain improvement appears to be one of the most important factors that has contributed the greatest profits (Nwachukwu *et al*., 2008. There are several factors which have an important impact on ethanol production, including the ability of yeast to tolerate stress factors such as heat shock, oxidative stress, osmotic stress, nutrient shortage, ethanol stress, pH down shift and anaerobic downshift. Yeast respond to various stresses in different ways; generally in *S. cerevisiae* there are 2 major stress response pathways. The first is known as the heat shock response which is mediated by heat shock transcription factors (HSF) (Morimoto *et al*., 1996) and the other is called the global stress response (GSR) which is occurred by a number of environment stresses such as nitrogen starvation, pH change, and oxidative stress (Schmitt and McEntee, 1996). One of the first stress factors that yeast cells must cope with is osmotic stress, caused by the high concentration of sugar at the beginning of the fermentation process. Osmotic stress occurs when an imbalance between intracellular and extracellular osmolarities occurs. This stress is believed to cause a deleterious change in the physiology of yeast (Csonka and Hanson, 1991 Response (HSR) helps yeast to respond to specific environment stresses (Morimoto *et al*., 1996). During fermentation, *S. cerevisiae* yeast cells generate heat during the process of converting glucose to ethanol. As a consequence, the fermentation temperature is usually controlled to ensure that yeast cells are not killed by elevated heat (Ingledew, 2009). Yeast growth and ethanol production can be affected in both positive and negative ways by temperature. According to Serra *et al*. (2005). If the temperature is increased from below optimum to optimum the yeast growth rate will increase. This is because biochemical reactions take place faster at higher temperatures and therefore the time and cost for fermentation

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can be reduced. However, high temperatures can cause negative effect to yeast, leading to inhibit cell viability which can have a disastrous effect on fermentation efficiency. Once the temperature exceeds the optimum temperature for the strain, the yeast growth rate decreases rapidly since the cellular membrane becomes damaged and essential enzymes for cellular metabolism are denatured (Serra *et al*., 2005). High temperature is responsible for stuck fermentations as yeast stress tolerance decreases at high temperature (Laluce *et al*., 1991; Thomas *et al*., 1993). In response to elevated temperature, yeast cells exhibit a rapid molecular response which is known as the Heat Shock Response (HSR). It is known that even sub lethal heat shock treatments can induce the synthesis of specific proteins commonly named as heat shock proteins (Hsps). In yeast, many Hsps perform molecular chaperoning functions which prevent protein aggregation while other Hsps can help yeast to be thermotolerance (Parsell and Linquist, 1994).

For example, in *S. cerevisiae*, Hsp104 plays a role in protecting yeast against heat denaturation and high temperature during respiratory growth (Lingquist and Kim, 1996). Hsp gene expression is known to be involved in the increased transcription of genes containing promoters of the heat shock element (HSE). The increase in gene transcription occurs due to the activation of heat shock transcription factor (HSF) in the presence of heat in order to allow yeast reproduction at high temperatures (Ruis and Schuller, 1995). Another response of yeast to high temperature stress is the accumulation of protective compounds such as glycerol (Omori *et al*., 1996), and enzymes such as mitochondrial superoxide dismutase and catalase (Costa *et al*., 1993).

However, arguably the most important protective compound for thermotolerance is trehalose (Van Laere, 1989; Wiemken, 1990; Neves and Francois, 1992). Trehalose is produced in response to the GSR pathway and also in direct response to heat shock, and helps to protect yeast by stabilizing cell membranes, as well as increasing the temperature stability of yeast cellular proteins. Typically, trehalose functions to replace water molecules in cellular membranes and forms a hydration shell around proteins (Iwahashi *et al*., 1995). Moreover, trehalose acts synergistically together with Hsp104 to confer thermoprotection (Elliot *et al*., 1996). The scope of this study is isolate therotolerant *S. cerevisiae* yeasts strains could become promising candidates for efficient industrial production of bioethanol throught heat shock.

MATERIALS AND METHODS

Sugarcane Molasses

Molasses sample (Brix. 85.6 total sugars 53.2%, fermentable 48.1% and un fermentable sugars 5.10%) was supplied by Egyptian sugar and integrated industries Company (ESIIC)

Yeast Strains

Saccharomyces cerevisiae strains were isolated from different sources namely sugar cane molasses of various Egyptian sugar factories, fermented molasses before distillation, sugar cane bagasse, banana peels as well as commercial active dry yeasts and maintained on PDA medium in Microbial Chemistry Lab. National Research Centre, Dokki, Giza, Egypt.

Inoculum Preparation

Sterilized 500 ml capacity conical flasks each contained 200 ml of medium containing (g /L) malt extract,3,yeast extract,3, peptone , 5 and sucrose,30 was steam sterilized at 121°C for 20 minutes, cooled to room temperature, then inoculated with a loop of yeast strains and incubated statically at 34°C for 24 hrs, The growing yeast biomass were used to inoculate experimental vessels at 34°C for 24 hrs. (Fadel *et al*., 2013)

Preparation of molasses medium

The sugar cane molasses was diluted with tap water. The previous diluted molasses supplemented with 0.5 g/l urea and 1 g/l diammomium phosphate as a source for nitrogen and phosphorus and 0.5 g/l magnesium sulfate. Amoxicillin+ flucloxacillin (1:1)was added as bacterial control according to Fadel *et al*.(2018). Molasses medium was dispensed into 200 ml capacity screwed bottles contained 100 ml of the molasses medium fermentation was carried out under non septic condition and incubated statically.

Screening of yeast strains

yeast strains were cultivated in diluted sugar cane molasses medium contained 15% w/v fermentable sugars dispensed into 200 ml capacity screwed bottles contained 100 ml. The fermentation was carried out under non septic condition and incubated statically for 36 hrs at 32^{o} C.

Complete fermentation of sugar cane molasses by selected yeast strains

Fourteen selected yeast strains produced the promising level ethanol yield were cultivated in diluted sugar cane molasses medium contained 15% w/v fermentable sugars dispensed into 200 ml capacity screwed bottles contained 100 ml. The fermentation was carried out under non septic condition and incubated statically for at 34^{o}C for 48 hrs.

Select of osmotolerant yeast strains

Seven selected yeast strains produced the highest levels ethanol yield from the previous step were cultivated in diluted sugar cane molasses medium contained 15%, 18, 20 and 22%w/v fermentable sugars dispensed into 200 ml capacity screwed bottles contained 100 ml .The fermentation was carried out under non septic condition and incubated statically for at 34^{o}C for 40 hrs.

Select of osmo tolerant faster fermentation yeast strains

Five yeast strains selected as osmo tolerant yeast strains from the pervious step were cultivated in diluted sugar cane molasses medium contained 20% w/v fermentable sugars dispensed into 200 ml capacity screwed bottles contained 100 ml .The fermentation was carried out under non septic condition and incubated statically hrs at 34^{o}C.

Heat shock

Three yeast strains Selected as osmo tolerant faster yeast strains were cultivated in sterilized 250 ml capacity conical flasks each contained 50 ml of medium containing (g /L) malt extract, 3, yeast extract,3, peptone , 5 and sucrose,30 was steam sterilized

at 121°C for 15 minutes, cooled to room temperature, then inoculated with a loop of yeast strains and incubated in rotary shaker 150 rpm at 34°C for 24 hrs. The three yeast strains were subjected to heat treatment i.e 43°C for 18 hrs, 50°C for 2hrs, 53o C 55°C for 20 minutes 55 °C for 30 minutes55°C for 2hrs.At the end of Heat treatment one ml of each was used to inoculate sterilized 250 ml capacity conical flasks each contained 50 ml of medium containing (g /L) malt extract,3,yeast extract,3, peptone , 5 and sucrose,30 and incubated in rotary shaker 150 rpm at 34°C for 24 hrs. The growing yeasts were used to inoculate the experimental bottles contained20% w/v fermentable sugars cane molasses medium dispensed into 200 ml capacity screwed bottles contained 100 ml. The fermentation was carried out under non septic condition and incubated statically for 48 hrs at 28,30,32, 34,36,38, 40,42and 44°C.

Analytical determination: Determination of un fermentable sugar (US) as residual sugars in fermented mash: The sugar concentration was determined by Fehling's titrimetric method (Lane and Eynon, 1923).

Estimation of ethanol content of the fermented mash

Ethanol content of the fermented samples was measured by ebulliometer approved in distillation factories (Fadel *et al*., 2014).

Fermentation efficiency

Fermentation efficiency was calculated as: ethanol yield in fermentation mash divided on theoretical ethanol yield multiply by 100.

Residual fermentable sugars

Residual fermentable sugars was calculated as: (100- Fermentation efficiency %) multiply by initial sugars percent.

RESULTS

Screening of yeast strains: Data presented in Table (1) show the ethanol yield of ninety *Saccharomyces cerevisiae* yeast strains cultivated on sugar cane molasses medium contained 15% w/v fermentable sugars after 48 hrs at 32°C.

Table1. Screening of *S. cerevisiae* yeast strains for the fermentation of sugar cane molasses for the production of ethanol

No	Code	Et OH % *	No	Code	Et OH %*	No	Code	Et OH %*
1	FH1	7.60	31	FNG43	0.85	61	F101	6.4
2	FH-96	7.75	32	NG43	1.65	62	F01X	5.70
3	FH3	7.30	33	NG43	3.45	63	F404	6.30
4	FH-4	7.75	34	FD24	5.70	64	4073	6.85
5	FH5	5.70	35	FD25	4.35	65	421	2.61
6	FH001	7.15	36	FD22	5.25	66	422	2.85
7	FH111	7.75	37	F25(11	5.40	67	425	2.15
8	FH222	7.60	38	F2511	6.10	68	F-81	0.00
9	FB3	2.15	39	FD20	5.75	69	F-8 2	0.55
10	FB2	2.00	40	F259(5	6.10	70	F-83	0.20
11	FB4	2.75	41	DF	6.85	71	F-84	0.75
12	FD1	7.00	42	DF25	6.00	72	F-85	1.10
13	FD3	6.10	43	DF30	7.00	73	M-50	5.75
14	FD2	6.30	44	DF32	6.70	74	M-50	6.85
15	FD4	6.00	45	E401	6.55	75	M-50	6.30
16	FD5	6.10	46	E402	6.70	76	M-54	2.50
17	FET1	6.40	47	E406	6.30	77	M- 58	2.60
18	FET-2	7.00	48	E257	4.65	78	543	2.00
19	FET-5	6.10	49	FE403	7.00	79	505	5.85
20	FET-3	7.00	50	E404V	5.25	80	549	2.00
21	FET-4	6.30	51	FKO	6.85	81	OXO	6.30
22	F404(1	6.30	52	FK1	0.00	82	266	2.50
23	F404	7.00	53	FK75	3.90	83	F-28	7.75
24	F405	6.70	54	FK25	6.00	84	268	2.60
25	FAL44	5.40	55	FK12	5.70	85	NE4	2.15
26	FAL44	6.00	56	FXXX	5.15	86	426	2.70
27	FAL44	6.00	57	FFX11	5.40	87	150	4.90
28	FAL44	4.90	58	FX100	6.85	88	50C1	6.00
29	FAL44	6.00	59	FX102	5.60	89	250	6.00
30	FNG43	1.30	60	FX105	4.90	90	350	5.60

***Ethanol yield**

Table 2. Fermentation of sugar cane molasses15% w/v fermentable sugars by 14 selected *S.cerevisiae* yeast strains after 72 h at 34o C

No	Strain Code	Et OH v/v*	Fermentation Efficiency %	Residual sugars%
1	FH-1	8.35	92.78	1.083
2	FH-96	8.75	97.22	0.417
3	FH-3	8.65	96.11	0.5835
4	FH-4	8.70	96.67	0.4995
5	FH-001	8.20	91.11	1.3335
6	FH-111	8.60	95.56	0.666
7	F-50	8.80	97.78	0.333
8	F-D1	8.60	95.56	0.666
9	FET-2	8.00	88.67	1.6995
10	FET-3	8. 00	88.67	1.6995
11	F-404	8.00	88.67	1.6995
12	FE-403	8.80	97.78	0.333
13	DF-30	8.00	88.67	1.6995
14	F-28	8.45	93.89	0.9165

*Ethanol yield

The yeast strains yielded ethanol over 7 % v/v(14 strains) FH-1, FH-96, FH-3, FH-4, FH-001, FH-111, FH-222 FD-1,FET-2, FET-3,F-404., DF-30,FE403., F-28, that bolded in Table 1 were selected to the following study. The selection based on, at industrial ethanol production from economic view high ethanol yield reduce the cost of energy demand for distillation consequently reduced the cost of the ethanol production.

Complete fermentation of sugar cane molasses by selected *S. cerevisiae* yeast strains at temperature 34° C

strains cultivated on sugar cane molasses medium 15% w/v fermentable sugars after 72 h at 34 °C .Data presented in Table (2) show the ethanol yield, residual sugars and fermentation efficiency of 14 selected strains from the above step up to complete fermentation.

Table 3. Fermentation of sugar cane molasses medium contained different fermentable sugars concentrations by seven selected S. cerevisiae yeast strains after 48 h at 34o C

Strain Code	15		18		20		22	
	EtOH%*	Effec.%**	EtOH%*	Effec.%**	EtOH%*	Effec.%**	EtOH%*	Effec.%**
FH-1	8.65	96.11	10.33	95.65	11.42	95.12	10.86	82.27
FH-96	8.75	97.22	10.48	97.04	11.63	96.67	11.44	86.22
F-50	8.70	97.78	10.52	97.41	11.60	96.62	11.20	84.85
FH-111	8.70	96.67	10.40	96.30	11.38	94.83	10.64	8o.61
F-D1	8.60	95.56	10.12	93.70	10.78	89.83	9.60	82.83
FE-403	8.80	97.78	10.54	97.59	11.64	97.00	11.51	87.20
F-28	8.60	95.56	10 .24	94.81	10.86	90.05	10.22	77.42

*Ethanol yield **Fermentation efficiency

Table 4. Fermentation of sugar cane molasses medium contained 20 % w/v t fermentable sugars concentrations by selected five osmotolerant *S. cerevisiae* yeast strains for different fermentation periods at 34o C

Strain Code	Fermentation time(hr)								
	20			40			60		
	R S %**	Effec .%***	Et OH %*	R S %**	Effec .%***	Et OH %*	R S %**	Effec .%***	Et OH %*
FH-1	7.38	7.70	61.50	10.12	3.13	84.33	11.38	1.03	94.83
FH-96	8.22	6.30	68.50	11.63	0.62	96.92	11.42	0.97	95.17
F-50	8.9	5.17	74.17	11.6	0.67	96.67	11.6	0.67	96.67
FH-111	7.46	7.57	62.17	9.96	3.40	83.00	11.46	0.90	95.50
FE-403	8.68	5.53	72.33	11.64	0.60	97.00	11.6	0.67	96.67

Table 5. Effect of heat shock for selected *S. cerevisiae* yeast strains on the ethanol yield at 40 oC

Strain	°C Heat treatment	Time	Before heat treatment			After heat treatment		
			EtOH%*	R S % **	Effec.% ***	EtOH%*	R S% **	Effec.% ***
	43	18hrs	9	75.00	5.00	10.35	86.25	2.75
	50	2hrs	9.4	78.33	4.33	10.95	91.25	1.75
	53	2hrs	9.2	76.67	4.67	10.15	84.58	3.08
FH-96	55	20min.	9.4	78.33	4.33	11.1	92.5	1.50
	55	30min.	9.65	80.42	3.92	11.42	95.17	0.97
	55	2hrs	9	75.00	5.00	10.55	87.92	2.42
	43	18hrs	9.45	78.75	4.25	11.55	96.25	0.75
	50	2hrs	9.1	75.83	4.83	10.25	85.42	2.92
F- 50	53	2hrs	9.35	77.92	4.42	10.85	90.42	1.92
	55	20min.	9.2	76.67	4.68	9.8	81.67	3.67
	55	30min.	8.7	72.50	5.50	10.85	90.42	1.92
	55	2hrs	8.4	70.00	6.00	10	83.33	3.33
FE-403	43	18hrs	9.1	75.83	4.83	11	91.67	1.67
	50	2hrs	8.95	74.58	5.08	11.1	92.50	1.50
	53	2hrs	9.2	76.67	4.67	11.25	93.75	1.25
	55	20min.	8.90	80.00	4.00	11.64	97.00	0.60
	55	30min.	8.35	69.58	6.08	10.8	90.00	2.00
	55	2hrs	7.9	65.83	6.83	9.2	76.67	4.67

*Ethanol yield **Residual sugars ***Fermentation efficiency

Table 6. Fermentation of sugar cane molasses medium contained 20 % w/v fermentable sugars concentrations by selected three thermotolerant S. cerevisiae yeast strains at different temperatures o C

Temp. °C	FH-96			F-50			FE-403		
	EtOH%*	R S %**	Effec.%***	EtOH%*	R S %**	Effec.%***	EtOH%*	R S %**	Effec.%***
28	10.22	2.97	85.16	10.85	90.42	1.92	10.94	91.17	1.77
32	10.82	1.97	90.16	11.45	95.38	1.08	11.48	95.93	1.43
34	11.18	1.37	93.16	11.5o	95.83	0.83	11.54	96.17	0.77
36	11.42	0.97	95.16	11.55	96.25	0.75	11.64	97.00	0.60
38	11.42	0.97	95.16	11.55	96.25	0.75	11.66	97.17	0.57
40	11.42	0.97	95.16	11.55	96.25	0.75	11.64	97.00	0.60
42	8.4	6.00	70.00	8.35	69.58	6.08	7.9	65.83	6.83
44	3.9	13.50	32.50	4.16	34.67	13.07	2.2	18.33	16.33

*Ethanol yield **Residual sugars ***Fermentation efficiency

Seven strains i.e FH-1, FH-96,FH-3,FH-4, FH-111,F-50,FD-1 and FE-403gave ethanol yield performance in fermentation mash with high fermentation efficiency as well as low residual sugars The mentioned strains in the Table(2) were selected for further study in the following trails.

Selection of osmotolerant S. cerevisiae yeast strains

In selecting yeasts for the efficient production of fuel ethanol, investigators have set out certain requirements for these yeasts. These include being ethanol tolerant, osmotolerant, depending on process requirements (Stewart *et al*., 1984). One of the first stress factors that yeast cells must cope with is osmotic stress, caused by the high concentration of sugar at the beginning of the fermentation process. Data presented in Table (3) show the ethanol yield and fermentation efficiencies of seven yeast strains i .e FH-1, FH-96, F-50, FH-111,FD-1, FE403., F-28 cultivated in sugar cane molasses medium involved four different fermentable sugars concentrations namely 15.18,20 and 22% w/v to select the more strains can ferment high gravity molasses. strains can ferment high gravity molasses posses advantages in industrial application in ethanol produc tion factories .Data obtained revealed that, five strains i.e FH-1, FH-96, F-50, FH-111and FE-403.produced performance ethanol yield in fermentation mash with high fermentation efficiency when cultivated in sugar cane molasses medium contained 20% w/v fermentable sugars for 40 hrs at 34^{o}C.

Selection of osmotolerant faster fermentation *Saccharomyces cerevisiae* yeast strains

The fermentation period one of the parameters must take in consideration in distilleries factories from economic view. Data presented in Table (4) show the ethanol yield, residual sugars and fermentation efficiency of 5 selected strains FH-1, FH-96, F-50, FH-111and FE403 from the above step to select the faster strain(s) can utilize fermentable sugars in sugar cane molasses to produce ethyl alcohol .Data reveal that strains FH-96, F-50 and FE-403 produced ethanol yield after 20 hrs as well as achieved its maximum performance in ethanol production after 40hrs. FH-1 and FH-111. gave less ethanol production efficiency .so less ethanol yield after 20hrs and achieved its maximum performance in ethanol production after 60hrs.

Effect of heat shock for selected S. cerevisiae yeast strains on the ethanol yield at 40 ^{o}C

Data presented in Table (5) show the effect of heat treatments the ethanol yield, residual sugars and fermentation efficiency of 3 selected osmololerant faster fermentation yeast strains i.e. *S. cerevisiae* FH-96 *S. cerevisiae* F-50 and *S. cerevisiae* FE-403 gave strains from the above step to select the faster strain(s) utilize fermentable sugars in sugar cane molasses to produce ethyl alcohol. Data reveal that, ethanol production was enhanced by the selected strains after heat shook comparable to ethanol yield before treatment when the fermentation was occurred at high temperature, and this reflect positively on the residual sugars and fermentation efficiency.

Fermentation of sugar cane molasses medium contained 20 % w/v fermentable sugars concentrations by three selected thermotolerant S. cerevisiae yeast strains at different temperatures

Data presented in Table (6) show the manor of the thermotolerant yeast strains namely. *S. cerevisiae* FH-96, *S. cerevisiae* F-55 and *S. cerevisiae* FE-403 for ethanol fermentation of sugar cane molasses medium contained 20 % w/v fermentable sugars concentrations at different incubation temperatures ranging from 28- 44^{o}C.Results revealed that *S. cerevisiae* FH-96 achieved its high fermentation efficiency at temperature 34-40^{o}C and both *S. cerevisiae* F-50and *S. cerevisiae* FE-403achieved its high fermentation efficiency at temperature between 32-40^{o}C.

DISCUSSION

From the genetic point of view, it is well known that the undesired mutation occur at high rate giving rise to degeneration of the strains industrial importance therfore strain instability is a constant problem in industrial utilization of microorganisms (Borkovich *et al*.,1989). The application of high-potential thermotolerant yeasts is a key factor for successful ethanol fermentation at high temperatures**.** Tolerance to high temperature is a desired feature in the yeast used in tropical countries like Egypt, in summer season where the temperature exceeds the optimum s for growth mesophilic microorganisms. In Egypt during the summer the temperature reaches of 40 ° C or more, which, combined with the heating of the fermentation due to the exothermic metabolic reactions makes the temperature in fermentors more than 40 ° C, causing heat stress and decreased productivity of ethanol. The use of thermotolerant yeast can minimize the thermophilic process as it avoids the use of expensive cooling systems (Laluce, 1991). In addition, avoids low ethanol efficiency production due to overheating, controlling of infection and minimizes the volume of wastewater generated in the distilleries (Banat *et al*., 1998 and Abdel -Fattah *et al*.,2000).

The phenomena associated with thermal tolerance (Laluce, 1991) and tolerance to ethanol (Ingram and Butke 1984, D'Amore *et al*., 1991). It has been reported that tolerance to ethanol and high temperatures are interactive. High concentrations of ethanol decreased the optimum temperature for growth. Various attempts to obtained thermotolerant strains of *Saccharomyces cerevisiae* i.e mutation, genetic engineering (Zhao and Bai, 2009). Jung and Park (2005), protoplast fusion, radiation, natural selection(Abdel -Fattah *et al*., 200, Fadel *et al*.,2013, Fadel *et al*., 2018) or subjected to thermal stress stress (DihaziI *et al*., 2001, Klipp *et al*., 2005). When we sought to isolate thermotolerant strains of *S. cerevisiae* the isolate mainly must have some futures, one of the first stress factors that yeast cells must cope with osmotic stress, caused by the high concentration of sugar at the beginning of the fermentation process. This stress is believed to cause a deleterious change in the physiology of yeast (Csonka and Hanson, 1991). Hyper-osmotic stress in which a high external osmotic pressure (for example a high concentration of soluble compounds) causes water inside the cell to be drawn out into the environment (Blomberg and Adler, 1992, Csonka and Hanson, 1991, DihaziI *et al.,* 2001, Klipp *et al.,* 2005).

Consequently at the start of fermentation hyperosmotic stress occurs due to the high concentration of sugars, and once sugars begin to be depleted hypo-osmotic stress may occur. It was reported that hyperosmotic stress can lead to the deterioration of viability, growth and fermentation performance (D'Amore, 1992). Further studies also support this finding, indicating that a loss of yeast viability may occur due to the reduction of intracellular water (Cahill *et al.,* 2000, Dumont *et al.,* 2003). In

response to elevated temperature, yeast cells exhibit a rapid molecular response which is known as the Heat Shock Response (HSR). Two genes in *Saccharomyces* HSp82 and HSC82 encode the HSP90 chaperone (Borovich *et al*., 1989 and Farrelly and Finkelstine 1984). The former gene is expressedat very low levin unstressed cells but highly induced during stress. HSC82 gene, on the other hand, is abundantly expressed at all times and only slightly induced during heat shock. Deletion of either HSP90 gene does not lead to detectable phenotype, but deletion of both genes together is lethal at all temperatures. (Borkovich, *et al*., 1989). It is known that even sub lethal heat shock treatments can induce the synthesis of specific proteins commonly named as heat shock proteins (Hsps). In yeast, many Hsps perform molecular chaperoning functions which prevent protein aggregation while other Hsps can help yeast increase thermotolerance (Parsell and Linquist, 1994).

For example, in *S. cerevisiae*, Hsp104 plays a role in protecting yeast against heat denaturation and high temperature during respiratory growth (Lingquist and Kim, 1996), whilst Hsp83 acts as a chaperone to prevent potential damage from protein misfolding caused by heat (Walker, 1998). Hsp gene expression is known to be involved in the increased transcription of genes containing promoters of the heat shock element (HSE). The increase in gene transcription occurs due to the activation of heat shock transcription factor (HSF) in the presence of heat in order to allow yeast to grow at high temperatures (Ruis and Schuller, 1995). It must be noted that HSEs only respond to heat shock and not to other stresses, and as such is a different kind of response to the Global Stress Response (GSR) pathway although several products of these pathways are similar, Another response of yeast to high temperature stress is the accumulation of protective compounds such as glycerol (Omori *et al.,* 1996), and enzymes such as mitochondrial superoxide dismutase and catalase (Costa *et al.,* 1993). However, arguably the most important protective compound for thermotolerance is trehalose (Van Laere, 1989; Wiemken, 1990; Neves and Francois, 1992). Trehalose is produced in response to the GSR pathway as well as in direct response to heat shock, and helps to protect yeast by stabilizing cell membranes, beside increasing the temperature stability of yeast cellular proteins. Typically, trehalose functions to replace water molecules in cellular membranes and forms a hydration shell around proteins (Iwahashi *et al.,* 1995). Moreover, Elliot *et al*. (1996) have found that in *S. cerevisiae*, trehalose acts synergistically together with Hsp104 to confer thermo-protection. There are many genes involved in trehalose synthesis including *TPS1, TPS2, TSL1, TPS3* as well as trehalose degradation such as *NTH1, NTH2, ATH1* (Zahringer *et al.,* 2000). These genes are regulated by STRE elements (Winderickx *et al.,* 1996) and are up-regulated in response to several stresses including heat stress and oxidative stress (Parrou *et al.,* 1997). While the synthesis of trehalose plays an important role in protecting cells. Indeed, trehalose is known to inhibit the activity of enzymes such as glutathione reductase, an enzyme which helps yeasts reduce oxidative damage within the cell and maintain cellular omeostasis (Sebollela *et al.,* 2004).

Conclusion

In the present study, a total of ninety strains of yeast were isolated from the various sources. Of these, three strains namely. *cerevisiae* F-96, *S. cerevisiae* F-50 and *S. cerevisiae* FE-403 were found to be highly thermotolerant after subjecting to heat shock to be fit for ethanol fermentation for high gravity sugar cane molasses (20%w/v) at 40 ^{o}C and high ethanol producer The ethanol yield 11.42 % v/v, 11.55 % v/v and 11.64 at 40 °C were achieved by *S. cerevisiae* F-96, *S. cerevisiae* F-50 and *S. cerevisiae* FE-403respectively. Furthermore, more than 96 % fermentable sugars utilization was achieved .The fermentation efficiency was between 95-97% .Thus, the three novels isolates could become promising candidates for efficient industrial production of bioethanol from Egyptian sugar cane molasses specially in summer season saving cooling system costs as well as increasing the efficiency of distillery factories

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