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OSMO-, THERMO- AND ETHANOL- TOLERANCES OF *SACCHAROMYCES CEREVISIAE* S_1

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ABSTRACT

Saccharomyces cerevisiae S_1, which is a locally isolated and improved strain showed viability at 40, 45 and 50°C and produced ethanol at 40, 43 and 45°C. When the cells were given heat shock at 45°C for 30min and grown at 40°C, 100% viability was observed for 60h, and addition of 200gL^{-1} ethanol has led to complete cell death at 30h. Heat shock given at 45°C (for 30min) has improved the tolerance to temperature induced ethanol shock leading to 37% viability at 30h. When the cells were subjected to ethanol (200gL^{-1} for 30 min) and osmotic shock (sorbitol 300gL^{-1}), trehalose contents in the cells were increased. The heat shocked cells showed better viability in presence of added ethanol. Soy flour supplementation has improved the viability of *S. cerevisiae* S_1 to 80% in presence of 100gL^{-1} added ethanol and to 60% in presence of 300gL^{-1} sorbitol. In presence of sorbitol (200gL^{-1}) and ethanol (50gL^{-1}) at 40°C, 46% viability was retained by *S. cerevisiae* S_1 at 48h and it was improved to 80% by soy flour supplementation.

Key words: Thermo-tolerance, ethanol-tolerance, osmo-tolerance, viability *Saccharomyces cerevisiae*

INTRODUCTION

The tolerance of yeast to its substrate (osmo-tolerance), fermentation product (ethanol- tolerance) and temperature (thermo-tolerance) has great potential to be used in industrial scale fermentation. At the beginning of fermentation, cells are subjected to high substrate concentration and as the ethanol level increases, both the substrate and product causes stress to the organism (14). During fermentation, heat is liberated due to exothermic reactions and if the environmental temperature is already high, the fermenter temperature tends to increase (1). Therefore the yeast should have temperature-, osmotic pressure- and ethanol- tolerating capacities to perform efficiently in industrial scale. In tropical countries like Sri Lanka, maintaining the operating temperature at or around the optimum fermentation temperature requires cooling, which is expensive. Significant cost savings become apparent if the fermenter can be kept at or above 40°C. In addition, ethanol recovery cost shall also be low if the process is carried out at higher temperatures. This however would require a yeast strain that could produce high titre of ethanol at higher temperatures.

Good yeast strain should have osmo-, ethanol- and combined ethanol and osmo-tolerances along with thermo-tolerance properties. Therefore this study was undertaken to evaluate the tolerance levels and fermentative capacities of a locally isolated and improved organism, *Saccharomyces*

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cerevisiae S_1 (2) up to 45°C for its application in local distilleries.

MATERIALS AND METHODS

Materials

Soy bean from local market was powdered and dried at 80°C. All the other materials were purchased from standard suppliers: culture media Oxoid limited USA, and other chemicals are from Sigma-Aldrich, USA.

Saccharomyces cerevisiae S_1

Saccharomyces cerevisiae S_1 is a locally isolated and improved thermotolerant strain (2); maintained in peptone, yeast extract and nutrient (PYN) – agar (2.5gL^{-1}) slants.

Analytical methods

Glucose (23), trehalose (TCA soluble anthrone positive carbohydrate) (36), ethanol (39) and viable cell count (30) were determined by standard methods.

Peptone, yeast extract and nutrient (PYN) medium

The medium contained (gL^{-1}) peptone, 3.5, yeast extract, 3.0, $MgSO_4.7H_2O$, 1.0, KH_2PO_4, 2.0; and $(NH_4)_2SO_4$, 1.0 at pH 5.0. Under different experimental conditions, different amounts of glucose were added to the medium and represented as glucose (amount in gL^{-1}) – PYN medium (2).

Inoculum of *S. cerevisiae* S_1

Glucose (50gL^{-1}) – PYN medium (100mL) was inoculated with 2 loops full of *Saccharomyces cerevisiae* S_1 and incubated at 36°C for 18h with shaking at 150rpm.

Thermo- tolerance and ethanol production

S. cerevisiae S_1 grown at 36°C in glucose (50gL^{-1}) –PYN medium for 18h was incubated at 40, 45, 50 and 55°C separately in triplicates and viability was monitored. All the following treatments were done in triplicates. For the ethanol production studies, inocua (10%, v/v, 18h) were added to the glucose (100gL^{-1}) – PYN medium and incubated at 40, 43 and 45°C separately with shaking (150rpm).

Temperature shift cultivation on ethanol –tolerance

Culture of *S. cerevisiae* S_1 prepared at 36°C in glucose (50gL^{-1}) – PYN medium was given different treatments as shown in Table 1 and the viable cell count was monitored.

Table 1. *S. cerevisiae* S_1 culture grown at 36°C in glucose (50gL^{-1}) – PYN medium were given different treatments. After the different treatment the cultures were incubated at the indicated temperature.

Culture No.	Treatment		*Incubation Temperature (°C)
	Heat shock	Added ethanol (gL^{-1})	
1	-	-	36
2	-	200	36
3	-	-	40
4	-	200	40
5	45°C for 30min	-	40
6	45°C for 30min	200	40

Heat shock on trehalase content and thermo-tolerance

To 18h old *S. cerevisiae* S_1 grown at 36°C in glucose (50gL^{-1}) – PYN medium, heat shock was given by incubating at 45°C for 30min. Control did not have heat treatment. Then 1mL aliquots of the test and control cultures were mixed with 1mL normal saline (pre-equilibrated at 58°C) and incubated at 58°C for 5min. The viability was determined. Trehalose was extracted (37) and estimated (36). Yeast cells without heat shock were used as control. Weight of the dry cells was measured.

Ethanol shock on trehalose content

Ethanol content in the *S. cerevisiae* S_1 culture grown for 18h at 36°C in glucose (50gL^{-1}) – PYN medium was measured and ethanol was added to make up the total concentration to 200gL^{-1}. After 30min, cells subjected to ethanol shock were harvested by centrifugation (7 x 10^3 rpm) and trehalose content and dry cell weight were measured. To the control, no ethanol shock was given.

Growth temperature on thermo-tolerance

S. cerevisiae S_1 inocula prepared at 28, 32 and 36°C were incubated at 58°C and viability was monitored. In another set-up 18 old culture grown at 28°C was incubated at 36°C for 90 min and then incubated at 58°C and the viability was monitored.

Soy flour supplementation on thermo-tolerance

Viability of *S. cerevisiae* S_1 grown at 40°C in glucose ($100gL^{-1}$) – PYN medium supplemented with $20gL^{-1}$ soy flour was monitored while the control medium did not have soy flour.

Soy flour supplementation on osmo-tolerance

Sorbitol ($0\text{-}400gL^{-1}$) was added to glucose ($100gL^{-1}$) – PYN. Soy flour ($40\ gL^{-1}$) was added to the test while the control did not have soy flour. Glucose ($200gL^{-1}$) – PYN medium and glucose ($300gL^{-1}$) – PYN medium with and without soy flour supplementation were also taken. Viable cell count and ethanol were determined at 48h of incubation.

Soy flour supplementation on ethanol tolerance

To glucose ($100gL^{-1}$) – PYN medium with and without soy flour ($40gL^{-1}$), ethanol ($0\text{-}200g\ L^{-1}$) was added and incubated at 40°C. Viable cell count and ethanol were measured at 48h.

Combined effects of osmo- and ethanol – stresses

Sorbitol ($200gL^{-1}$) was added separately into different medium prepared with and without soy flour and, viable cell count and ethanol were measured at 48h.

RESULTS AND DISCUSSION

Thermo-tolerance and ethanol production

When 18h old cultures of *S. cerevisiae* S_1 grown in glucose ($50gL^{-1}$) – PYN at 36°C were incubated at different temperatures, the organism retained 100% viability up to 72, 10.0, 2.0 and 0.5h respectively at 40, 45, 50 and 55°C and the organism lost 50% of its viability at 120, 66, 39 and 5h at the respective temperatures (Figure 1). Since the organism showed considerable viability at higher temperatures, its ethanol producing ability at 40, 43 and 45°C were studied. Temperatures above 45°C were not considered, as the fermentation processes do not exceed 42°C in large industrial level operations (1). The ethanol production at 40, 43 and 45°C in glucose ($100gL^{-1}$) – PYN medium was 46, 38 and $26gL^{-1}$ respectively (Table 2). D'Amore et al., (7) also have shown that the increase in the temperature from 40 to 45°C resulted in a decrease in the rate and extent of glucose utilization and ethanol production. Since *S. cerevisiae* S_1 was able to survive and produce ethanol from 40 to 45°C, the effect of heat stress on its ethanol tolerance was studied.

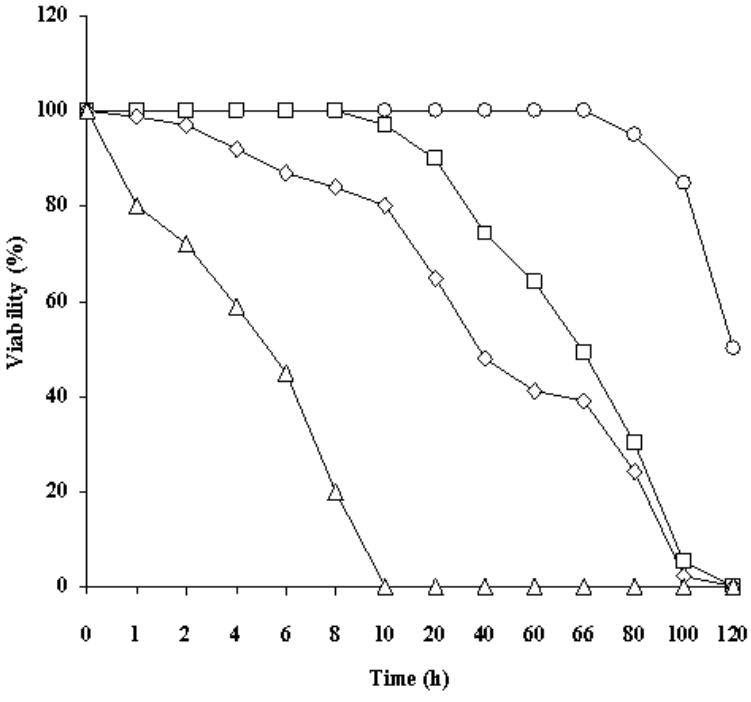

Figure 1. Viability of *S. cerevisiae* S_1 grown in glucose ($50gL^{1}$) – PYN medium at 36°C and the 18h old culture was subjected to heat treatment at (○) 40; (□) 45; (◊) 50 and (△) 55°C.

Table 2. Ethanol production by *S. cerevisiae* S_1 at different temperatures in glucose ($100gL^{-1}$) - PYN medium at 24h.

Temperature (^oC)	Ethanol (gL^{-1})	Residual sugar (gL^{-1})	Ethanol production efficiency (%)	Sugar utilization (%)
40	46	0.0	91.0	100.0
43	38	19.5	74.4	80.5
45	26	45.0	50.8	55.0

Ethanol production efficiency = Ethanol produced/ Theoretical maximum of ethanol that could be produced from initial glucose.
**Glucose utilization was calculated ad the percentage of glucose used from the initial amount of glucose added to the medium.

Temperature shift cultivation on ethanol – tolerance

When 18h old *S. cerevisiae* S_1 inoculum was given different treatments (Table 1) and grown at either 36 or 40°C, the viability of control cultures which had no treatments (heat shock or added ethanol) at either 36 or 40°C remained almost 100% (Figure 2). The heat shocked cells (at 45°C for 30 min) showed 100% viability when they were grown at 40°C without added ethanol. When $200gL^{-1}$ ethanol was added to the culture (18h) grown at 36°C, complete death of cells was observed at 60h, whereas at 40°C in presence of ethanol complete cell death occurred at 30h. Therefore the toxic effect of ethanol was aggravated by the increase in incubation temperature. However the temperature induced ethanol toxicity was nullified by a brief heat shock (30min) at 45°C. Heat shocked culture showed 37% viability at 30 h at 40°C in the presence of ethanol as against complete cell death of the cells, which were not given heat shock.

It was reported that ethanol had specific and non-specific effects directly on membranes and proteins (16). Heat shock leads to changes in the fatty acid composition of the membranes (22, 25), synthesis of heat shock proteins (5, 12, 17, 22, 40) and accumulation of trehalose (19, 38, 4, 11, 15, 26, 28, 29, 31, 32, 33, 42). To evaluate whether the heat shock induced ethanol tolerance in *Saccharomyces cerevisiae* S_1 was due to the accumulation of trehalose (TCA soluble anthrone positive carbohydrate), the changes in trehalose content along with heat shock and thermo-tolerance were studied.

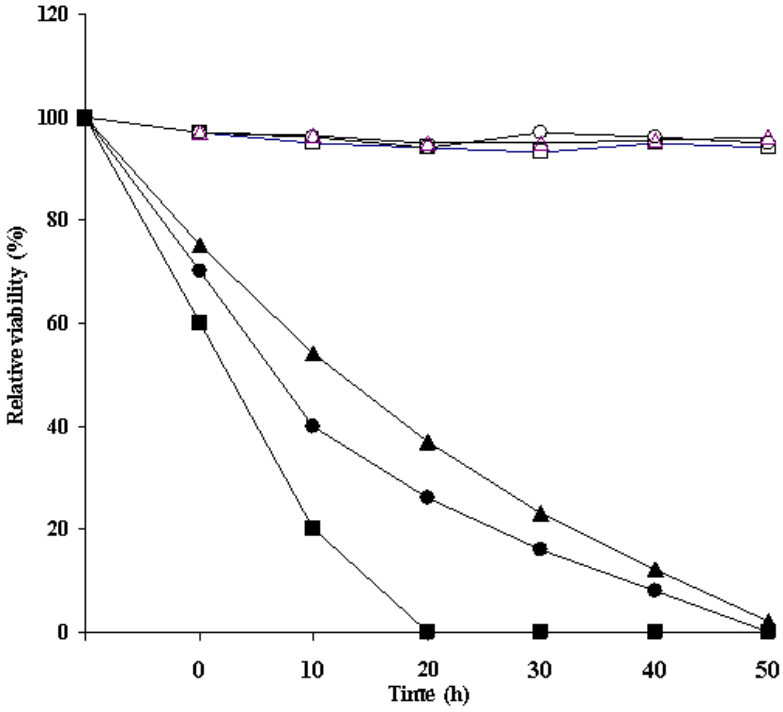

Figure 2. Effect of heat shock on ethanol tolerance of *S. cerevisiae* S_1. Inoculum was developed at 36°C and to the 18h old culture ethanol ($200gL^{-1}$) was added. Viability at (O) 36°C without ethanol; (●) 36°C with ethanol; (□) 40°C without ethanol; (■) 40°C with ethanol (△) at 40°C without ethanol after heat shock and (▲) at 40°C with ethanol after heat shock.

Heat shock on trehalose content and thermo-tolerance

Trehalose content was increased by 90.3% in heat shocked cells; while 28% viability was observed at 30h for heat shocked cells as against complete cell death for culture not undergone heat shock. Trehalose content was increased from 62 (±2.0) to 118 (±5.0) (µmole glc / g dry cell weight) after heat shock treatment. Therefore the increase in trehalose content could be linked to enhanced thermo- tolerance of *S. cerevisiae* S_1 cells as for other yeast (19, 28, 34) strains. Accumulation of enormous amounts of trehalose (28) could be due to increased levels of trehalose metabolism (42). Trehalose at first regarded only as an energy reserve (13), but latter studies have shown the correlation between the trehalose content of yeast cells and their resistance to temperature extremes (24, 28, 41), high osmotic pressure and high ethanol concentrations (18). Trehalose acts as a protecting agent to the cell membranes and proteins under conditions that deplete intracellular water (6, 9). Since the relationship between trehalose content and thermo-tolerance was observed in this study, the effect of ethanol stress on the trehalose content of the cells was also studied.

Ethanol shock on trehalose content

Trehalose content was 66 (±1.0) and 95 (±4.0) µmoles glucose equivalent/dry cell weight respectively in control and test samples. A 44% increase in trehalose content was induced by ethanol shock. Ethanol causes water stress by lowering water activity (a_w) and thereby interferes with hydrogen bonding within and between hydrated cell components leading to ultimate disruption of enzymes and membrane structure and function (8). Yeast cells exposed to ethanol, synthesize compatible solutes such as glycerol and trehalose as protectants (14). Ethanol induced leakage was inhibited by the accumulated trehalose (20, 26, 27). Under water stress, cells synthesize compounds that protect the structure and function of hydrated cell components, the compatible solutes and the trehalose formed show protective effects on membranes (28). When the ethanol shock as well as heat shock was studies individually, both studies have shown the induced accumulation of trehalose. In the mean time heat shocked cells showed better ethanol tolerance. The stress conditions have been circumvented by the production of trehalose. The accumulation of trehalose in the heat shocked cells was more than that in the ethanol stressed cells. Since the heat shock at 45°C for 30min has improved the viability of *S. cerevisiae* S_1, the effect of growth temperature on the thermo-tolerance of the organism was studied.

Growth temperature on thermo-tolerance

The cultures grown at different temperatures showed vast difference in thermo-tolerance at 58°C (Figure 3). In addition to growth temperature, heat shock prior to high temperature exposure had significant effect on thermo-tolerance. When the culture grown at 28°C were incubated at 36°C and was subjected to 58°C, 20% viability was observed, while the cells directly transferred from 36 to 58°C showed 2% viability at 10h.

The increase in the thermo-tolerance due to the rapid shift in growth temperature from 28 to 36°C resulted in protection from death at 58°C. When the growth temperatures were 23, 30 and 36°C, the survival fraction (%) of the *S. cerevisiae* at 52°C at 5min were 90, 10 and 0.8 respectively (7). In our experiment the cultures grown at 28, 32 and 36°C showed 1, 12 and 28% viability at 58°C after 5h. The shift in growth temperature from 28 to 36°C has resulted in significant increase in viability (50%). Viability of the cells at 58°C reached zero at 10 and 20 h with cultures grown at 28 and 32°C respectively. At 30min, 0.1 and 1.0% viability was observed with cultures grown at 36°C and heat shocked (28 to 36°C) cultures respectively. Shifting yeast culture from 23 to 36°C for a brief period would result in improved viability (22).

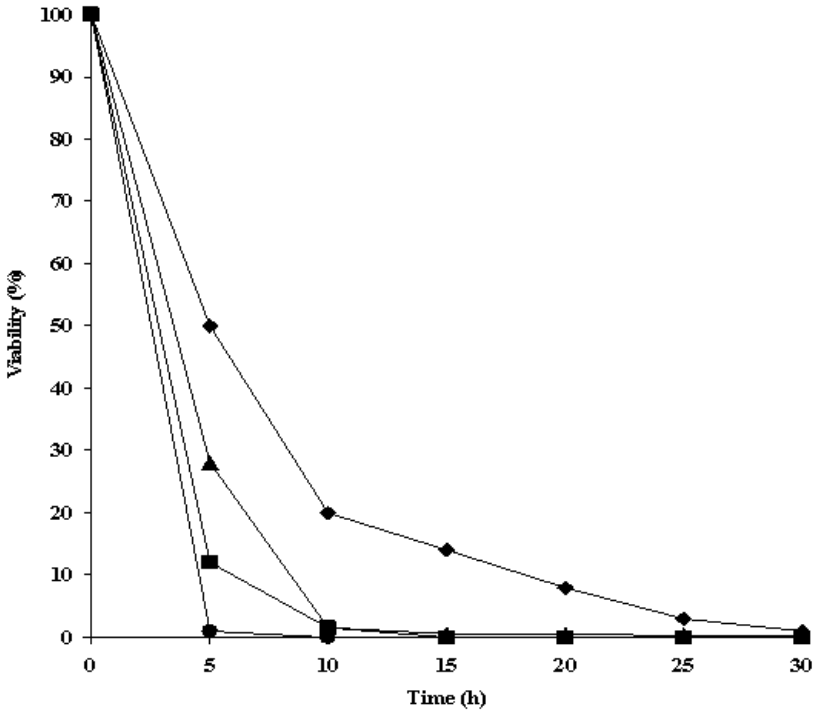

Figure 3. Viability of *S. cerevisiae* S_1 at 40ºC after the heat shock at 58ºC. The cells grown at (●) 28; (■) 32 and (▲) 36ºC, and (♦) grown at 28ºC incubated at 36ºC for 90min, prior to heat shock at 58ºC.

Soy flour supplementation on thermo-tolerance

Soy flour supplementation has improved the thermo-tolerance of *S. cerevisiae* S_1 at high temperature (Table 3). At 40°C, when soy flour was supplemented, the duration of 100% viability was extended from 72 to 86h. At 45°C the same was increased from 10 to 16h by soy flour supplementation. The soy flour supplement was not effective at 50 and 55°C. The increase in the viability at 40 and 45°C by soy flour supplementation suggests that *S. cerevisiae* S_1 cells required additional nutrients to withstand high temperature. Addition of 1% yeast extract and 1µmol oleic acid (mL medium^{-1}) has eliminated the initial death phase of *S. cerevisiae* at 40°C (42). Soybean flour contains 38% proteins and 21% lipids (44), and of the lipids appreciable amount (26%) of oleic acid is present (3). Therefore the effect of soy flour could be linked to its oleic acid content. Beneficial effects of soybean on viability of cells in high gravity molasses at high temperature was reported (10). One effect of heat is to induce nutritional requirements for lipids (44). Therefore improvement in thermo-tolerance by soy flour supplementation could be due to its unsaturated fatty acid content. Since soy flour has improved the thermo-tolerance of *S. cerevisiae* S_1, its effect on osmo- tolerance was determined.

Table 3. Viability of *S. cerevisiae* S_1 at different temperatures. The indicated times represent the maximum period to which the strain was able to retain 100% viability.

Medium	**Time (h)**			
	40°C	**45°C**	**50°C**	**55°C**
Glucose (100gL^{-1}) –PYN	72	10.0	2.0	0.5
Glucose (100gL^{-1}) –PYN + Soy flour (20gL^{-1})	86	16	3.0	1.0

Soy flour supplementation on osmo-tolerance

The osmo-tolerance of *S. cerevisiae* S_1 in presence of different concentrations of (0-400gL^{-1}) of non-metabolizable sugar sorbitol (44) was determined.

The viability and ethanol producing ability of *S. cerevisiae* S_1 were significantly not affected by sorbitol up to 100gL^{-1}. With 200gL^{-1} sorbitol (containing 100gL^{-1} glucose), the viability and the ethanol production were reduced to 60% and 30gL^{-1} respectively (Table 4). Further increase in sorbitol exerted severe effect on both viability and ethanol production. Therefore the results suggest that high sugar levels caused the death of the cells along with decreasing ethanol producing ability, i.e. both the aerobic and anaerobic metabolisms of the yeast were affected. Increase in solute concentration results in increased osmotic pressure and decreased water activity (14, 44). It has been previously reported that the increase in the osmotic pressure of the medium can decrease the viability and fermentative ability, in addition to the intracellular ethanol (44).

Supplementation of soy flour increased the viability and fermentative ability of *S. cerevisiae* S_1 at high sugar levels (Table 4). Therefore the additional nutrients support *S. cerevisiae* S_1 to combat the osmotic pressure and / or water stress.

The sugar utilization and ethanol production efficiency of *S. cerevisiae* S_1 are higher in sorbitol (100gL^{-1})- glucose (100gL^{-1}) – PYN medium than glucose (200gL^{-1}) – PYN (without sorbitol) (Table 5). However, when these media were supplemented with soy flour, increased sugar utilization and ethanol production efficiency were observed with glucose (200gL^{-1}) – PYN (without sorbitol) medium. The same is true when either 200gL^{-1} sorbitol or 200gL^{-1} glucose was added to glucose (100gL^{-1}) – PYN medium (Table 4). From the results it can be concluded that the inhibitory effect given by metabolizable sugar was more than that of non-metabolizable sugar and this effect could be rectified by adding soy flour (40.0gL^{-1}) to PYN medium. The studies showed that both viability and ethanol fermentative ability of *S. cerevisiae* S_1were affected by increasing the osmotic pressure and these effects were reduced by the soy flour supplementation. A study was carried out to find the tolerance property of the organism to both osmotic and ethanol stresses in presence of soy flour.

Table 4. The effect of soy flour (40 gL^{-1}) supplementation on osmo-tolerance, ethanol production, ethanol production efficiency (EPE) and sugar utilization under different conditions *by S. cerevisiae* S_1 cells in glucose (100 or 200 or 300gL^{-1}) – PYN medium at 48h and 40°C.

Glucose (gL^{-1})	Sorbitol (gL^{-1})	PYN				PYN + Soy flour			
		Viability (%)	Ethanol (gL^{-1})	Sugar utilization (%)	EPE (%)	Viability (%)	Ethanol (gL^{-1})	Sugar utilization (%)	EPF (%)
100	0	96	46	100	90.0	97	47.0	100.0	92.0
100	50	95	45	100	88.0	97	47.0	100.0	92.0
100	100	90	42	90	82.0	97	46.0	97.0	90.0
100	200	60	30	62	58.7	90	43.0	90.0	84.0
100	300	38	20	57	39.0	60	32.0	67.8	62.6
100	400	5	8	83	15.6	25	19.0	40.2	37.0
200	-	ND	75	79	73.3	ND	98.6	100.0	96.5
300	-	ND	82	60	53.5	ND	149	100.0	97.0

Viability (%) = (Viable cells / Total cells) x 100
ND – Not determine

Table 5. Effect of added ethanol concentration on viability of *S. cerevisiae* S_1 and ethanol production in glucose ($100gL^{-1}$) – PYN medium and in soy flour ($40\ gL^{-1}$) supplemented glucose ($100gL^{-1}$) –PYN medium at 40^oC and at 48h.

Ethanol added (gL^{-1})	PYN			PYN + Soy flour		
	Viability (%)	Final ethanol (gL^{-1})	*Ethanol roduced (gL^{-1})	Viability (%)	Final ethanol (gL^{-1})	*Ethanol produced (gL^{-1})
0	95	46	46	96	48	48
50	90	90	40	94	92	42
100	70	128	28	80	132	36
150	0	155	5	20	172	24
200	0	200	0	0	204	0

Viability (%) = (Viable cells / Total cells) x 100
*Ethanol produced = Final ethanol – added ethanol

Soy flour supplementation on ethanol-tolerance

Ethanol tolerance of *S. cerevisiae* S_1 was determined with different concentrations (0-$200gL^{-1}$) of added ethanol in glucose ($100gL^{-1}$) – PYN medium supplementation. The effect of soy flour supplementation on ethanol tolerance was also studied. The viability was reduced with the increased added ethanol concentration. Complete cell death was observed at 48h with $150gL^{-1}$ added ethanol (Table 5). With $100gL^{-1}$ added ethanol, the viability was improved from 70 to 80% with soy flour supplementation at 48h. With 150 and $200gL^{-1}$ added ethanol, complete cell death was observed in un-supplemented media and in soy flour supplemented media 20.0 and 0.0% viability was observed respectively. The results suggested that soy flour supplementation was ineffective at and above $200gL^{-1}$ added ethanol.

Since the soy flour seems to improve the osmo- and ethanol – tolerances individually another experiment was performed to see its effect on combined osmo- and ethanol – tolerances of *S. cerevisiae* S_1.

Combined effects of osmo- and ethanol – stresses

Combined effect of different concentrations (0-$200gL^{-1}$) of added ethanol and sorbitol ($200gL^{-1}$) on *S. cerevisiae* S_1 was studied in glucose ($100gL^{-1}$) – PYN and soy flour supplemented glucose ($100gL^{-1}$) – PYN media separately. With the increase in added ethanol amount to the glucose ($100gL^{-1}$) – PYN medium having $200gL^{-1}$ sorbitol, complete cell death was observed at $100gL^{-1}$ added ethanol, while 10% viability was recorded with the addition of soy flour (Table 6). In the glucose ($100gL^{-1}$) – PYN medium with $200gL^{-1}$ sorbitol, ethanol produced was 28 and $12gL^{-1}$ with 0 and $50gL^{-1}$ added ethanol respectively (Table 6). Hence the addition of ethanol has reduced ethanol production by 57%. However with soy flour supplementation, the ethanol produced was 42 (without added ethanol) and $30gL^{-1}$ (with $50gL^{-1}$ added ethanol). This is only a 30% decrease in ethanol production. Thus soy flour has increased the fermentative ability of the *S. cerevisiae* S_1 under combined osmotic- and ethanol – stresses. The viability also has been increased from 60 to 90 and 46 to 80 with soy flour supplementation at 0 and $50gL^{-1}$ added ethanol respectively. At higher ethanol levels (100 - $200gL^{-1}$) with sorbitol ($200gL^{-1}$), even the soy flour supplementation could not enhance the viability and fermentative capacity of yeast significantly. The combined effects of osmotic- and ethanol stresses were more pronounced than their individual effects (Table 6). The results suggest that there are additional complex nutritional requirements for the survival of *S. cerevisiae* S_1 under combined stressed conditions. During high gravity fermentation, initially high osmotic stress is experienced by the strain and as the fermentation proceeds the sugar depletes and the alcohol concentration increases. Hence as the fermentation proceeds, the strain undergoes ethanol stress. Therefore a strain with both ethanol- and osmo- tolerance is necessary to comply with the high gravity fermentation successfully.

Table 6. Viability of and ethanol produced by *S. cerevisiae* S_1 cells in presence and absence of (40 gL^{-1}) soy flour with increased concentration of added ethanol while maintaining the sorbitol at 200gL^{-1} level in glucose (100gL^{-1}) – PYN media at 40°C at 48h.

Ethanol added (gL^{-1})	#Viability (%)				Ethanol produced by fermentation (gL^{-1})			
	Without sorbitol		With sorbitol		Without sorbitol		With sorbitol	
	PYN	PYN + Soy flour	PYN	PYN + Soy flour	PYN	PYN + Soy flour	PYN	PYN + Soy flour
0	95	96	60	90	46	48	28	42
50	90	94	46	80	40	42	12	30
100	70	80	0	10	28	32	0	10
150	0	20	0	5	5	22	0	0
200	0	0	0	0	0	0	0	0

#Viability (%) = (Viable cells / Total cells) x 100

CONCLUSION

S. cerevisiae S_1 has shown viability and ethanol producing ability between 40 to 50°C and 40 to 45°C respectively. When the cells were given heat shock prior to inoculation, they were able to perform better and tolerate heat, ethanol and osmotic stresses by producing trehalose. The cells were able to tolerate thermo-, ethanol and osmotic stresses better in presence of soy flour than in the absence of soy flour. Therefore more detailed study has to be to be made to find the effect of soy flour on *S. cerevisiae* S_1.

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