

*Full Length Research Paper*

# **Influence of thermal and osmotic pressure on the growth and viability of *Candida shehatae var shehatae***

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**Environmental stresses can bring abrupt changes in microbes and it can affect its growth with a number of visible modifications. Although some studies emphasize on the bacterial and fungal cellular response against acute heat and mitochondrial substrate oxidative stresses, in case of yeast very few researches were conducted. Currently, *Saccharomyces cerevisiae* is only known yeast about their responses against different stresses. The present study has been performed to observe how the growth and budding pattern of *Candida shehatae var shehatae* will be influenced by high temperature (40-45°C), as well as the impact of osmotic stresses on yeast cell. To find out the critical growth temperature of *C. shehatae var shehatae* growth was monitored at 40 and 45°C for 72 h. Osmotic stress tolerance was observed at 32.5°C in diverse dextrose (0.04 g/l (1X), 0.12 g/l (3X), 0.2 g/l (5X), 0.28 g/l (7X), 0.36 g/l (9X)) and sucrose, that is 0.02 g/l (1X), 0.06 g/l (3X), 0.1 g/l (5X), 0.14 g/l (7X) , 0.18 g/l (9X) concentrations. The result was monitored at 600 nm at different time intervals. The viability of strains was analyzed by determination of CFU/ml. The optimal growth pattern was observed at 32.5°C and cells lose their cultivability at 45°C. Cell growth and budding were observed under high dextrose and sucrose concentrations and suppressed at 45°C. Results of the current study indicated that the critical growth temperature is 45°C and under sugar concentrations stress stimulant was also shown at that temperatures.**

**Keywords:** *Candida shehatae var shehatae*, dextrose, heat stress, critical growth, osmotic stress, morphological pattern.

## **INTRODUCTION**

*Candida* is categorized as a common cause of mycoses around the world; among the 154 species, some are pathogenic (Aggarwal et al., 2018). The growth properties and physiological potentiality of microbes are often emphasized in microbiology. Survivability rate of microbes depend on numerous factors like stresses that suppress

their growth potentiality (Haruta and Kanno, 2015). Incessant changing environment is a selective pressure and may induce genetic modification. The previous research reported that different altering environments affect the growth and function of microorganisms (Ikeda et al., 2014; Asako et al., 2013; Yamaguchi et al., 2014).

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For instance it has also been proposed that competitive relationships, stress tolerance, biological and physiological disturbance should have been considered as microbial life strategies (Krause et al., 2014). Microbes have great number of strategies to face stressful situations counting spores and cysts formation, stress reliever as molecule synthesis, changes in cellular membrane, expression of reconstructed enzymes for damage and so forth (Storz and Hengge, 2011). The salient factor in controlling the comprehensive reaction to elevated temperature and stages of reaction oxygen species are participated by transcriptional activation. Fluctuating nutrient levels, osmotic imbalance, non-optimal level temperatures, impact of toxic molecules are some stresses which are caused by those environmental changes (Morano et al., 2011). Yeast cells have the ability to sense the thermal stress; specific heat shock sensitive proteins can perform as a biological thermometer, but has some lack of evidence in eukaryotic systems (Craig and Gross, 1991). In a senescent stage microbes do not stop all metabolic activity, they stay metabolically dynamic to continue viability and defend itself against stress condition (Polymenis and Kennedy, 2017). Heat stress in microbes is a situation where too much heat is absorbed by it and causes stress. It can cause changes in the cell or can even lead to death. Another environmental stress condition is osmotic shock termed as physiologic dysfunction. It is responsible due to abrupt change around a cell in the solute concentration that can cause ascending change in water movement across its cell membrane. Temperature, the redox state, pH, toxic compounds, salt/sugar concentrations, which are instantaneous changes in environmental and physicochemical stimuli (Turkel, 2000; Mager and Varela, 1993), as well as nutrient enervation have been typically initiated in bacterial cells to evoke a series of defensive mechanism which encodes heat shock proteins by up-regulating the genes (Munna et al., 2015). DNA damage, cell shrinkage, or cell burst could be the result of physiological stress that occurs in the growth habitat of living organisms. *S. cerevisiae* is an organism in which gene regulation is affected dramatically by some physiological events like osmotic stress, heat shock and oxidative stress (Turkel, 2000). Oxidative stress can be generated externally by reactive oxygen species or indirectly in the final stages of the respiratory chain. These reactive oxygen species are responsible for generating the response pathway, though they repress the expression of the subset of genes (Jamieson, 1998; Jamieson et al., 1994). Like other yeasts, in *Candida* the heat shock response has also been noticed in the cellular physiology by the rapid changes, also seen in the budding behavior which is accompanied by the increased tolerance against raised sugar and salt concentrations. In another study, *S. cerevisiae* showed heat sensitivity which is usually prescriptive of imperfections in genes

that code proteins and also plays a fundamental role for maintaining the cell viability (Parsell et al., 1994). In yeast cells, cellular degeneration and retardation in cell division are influenced by heat stress and osmotic shock together (Rowley et al., 1993). In terms of local perspective, very few studies on stress response in yeast cells have been conducted. On the other hand, studies on bacterial stress responses have been conducted more times than on yeast (Święciło, 2016). However, the existence of fewer studies on the stress response to yeast cells led us to conduct the study of critical and optimal growth temperature, looking to the growth alterations at the decisive temperature and also with the stimulated stressed condition of rapid changes in osmotic pressure. The current study was conducted to find out the response of yeast *C. shehatae* var *shehatae* towards specific heat shock and high sugar concentrations. To our best information, this is the first inclusive report on a promising *C. shehatae* var *shehatae* strain in Bangladesh. The experimental outcomes will give novel insight about the combined effect of different stresses on *C. shehatae* var *shehatae*.

## MATERIALS AND METHODS

### Yeast and growth conditions

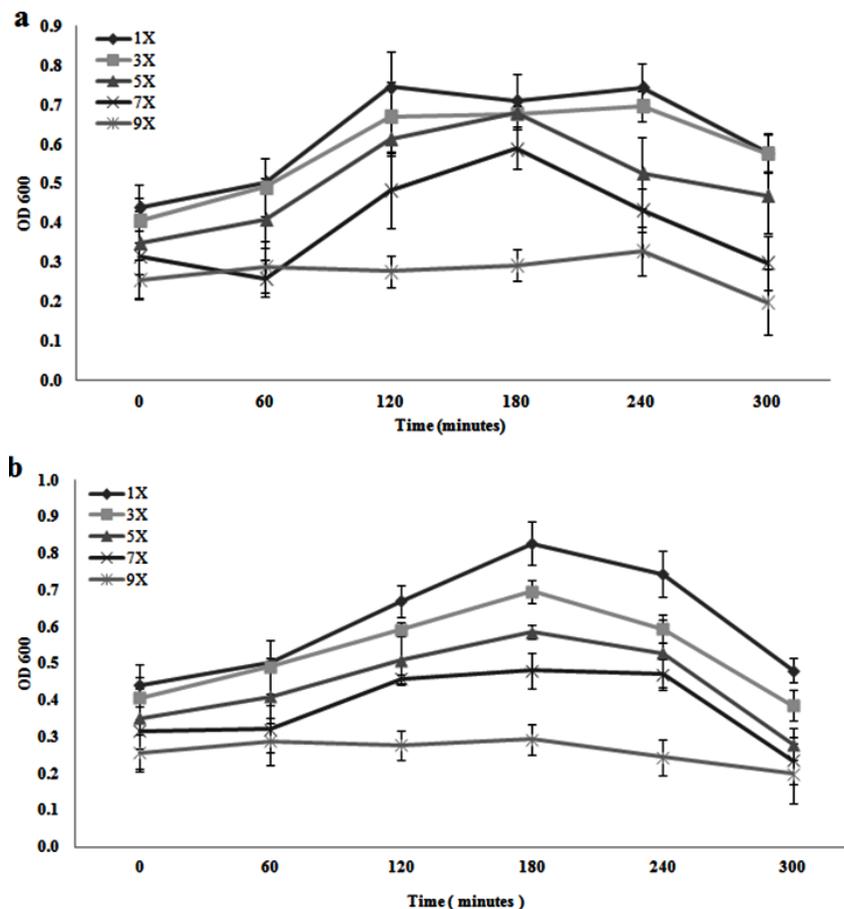
The yeast *C. shehatae* var *shehatae* was previously isolated and identified (Deposited as IM-101) in the Industrial Microbiology Laboratory, IFST, BCSIR, Dhaka. The yeast strain was grown in Sabouraud Dextrose Agar (SDA) medium (Hi-Media, India) and harvested for 48 h at the beginning of the stationary phase. Measurements of cell viability were carried out with the standard plate method on the SDA medium by suitable dilutions.

### Osmotic treatment

The Organism was freshly grown on SDA medium. A freshly prepared yeast culture plate was used from where a loopful of a colony was taken and inoculated in 5ml SDB, followed by incubation at 32.5°C for 72 h. Dextrose and sucrose were used for the spot dilution test in order to find out the osmotic tolerance (McKenzie et al., 2010). A spot dilution test were conducted by taking 1 ml of culture suspension and at 60 min interval, then serial dilution occurred up to 10<sup>-4</sup> in 9ml sucrose and dextrose broth. Then 1 ml of culture from every dilution was mottled onto SDA media plates and incubated for 24 h at 32.5°C (Nur et al., 2014). Observation of the osmotic effect on yeast cell growth, diverse concentrations of dextrose counting 0.04 g/l (1X), 0.12 g/l (3X), 0.2 g/l (5X), 0.28 g/l (7X), 0.36 g/l (9X) and sucrose, that is, 0.02 g/l (1X), 0.06 g/l (3X), 0.1 g/l (5X), 0.14 g/l (7X) and 0.18 g/l (9X) were employed. The ability to grow through colony forming units and the optical density at 600 nm (OD<sub>600</sub>) were monitoring at specific time intervals. From each of the culture suspension, an aliquot of 5µl was removed at 60 min intervals for morphological observation (Munna et al., 2015).

### Heat treatment

The yeast cultures were arranged by inoculating a loopful yeast



**Figure 1.** *C. shehatae var shehatae* assesment growth temperature through the examination, by measuring the absrbance at 600nm (a) after 48 h of incubation, (b) 72 h of incubation.

colony from the freshly prepared yeast cultures plate into 5 ml SDB, followed by incubation at 30, 35, 37, 40 and 45°C in standing state up to 72 h. The ability to grow was monitored by colony forming units' counts and optical density at 600 nm ( $OD_{600}$ ) at specific time intervals. From each of the culture suspension, an aliquot of 5 $\mu$ l was removed at 60 min intervals for morphological observation. Growth was observed at 40 and 45°C in order to determine the critical growth temperature. From each of the culture suspension, an aliquot of 5 $\mu$ l was removed at 60 min intervals for morphological observation (Munna et al., 2015).

#### Statistical analysis

For statistical analysis, all experiments were done in three replicates. Significance of the differences was assessed by the least significant difference (LSD) test for several comparisons of means of growth diameter. Results were presented as mean  $\pm$  SD.

## RESULTS

The optimal growth temperature for *C. shehatae var shehatae* was estimated by the measurement of  $OD_{600}$

along with sum up the CFU up to 300 min. Rapid increase of cell number was observed after 60 min of incubation at 32.5°C. On the other hand, at 30°C, propensity for growth was a bit steady. However, comparatively long lag phase (~120 min) was seen after the growth of the cells at 37 and 40°C, juxtaposed with the growth stage at 32.5 and 30°C. This conceivably was found due to the requirement of coping with the higher temperature for more time compared to the optimal growth conditions (Figure 1a). After 120 min at 30 and 32.5°C, budding yeasts like cells were noticed under the light microscope but similar budding was spotted after 180 min at 37°C. The budding occurrences at 40°C were noted after 240 min (Figure 3). CFU cell counts after 48 h of incubation showed highest growth at 32.5°C (Table 1). The most favorable growth temperature for *C. shehatae var shehatae* was 32.5°C. However, at 45°C when cells were grown, the budding yeasts were noticed to become dormant (Figure 3). Also at 45°C after 72 h growth on the plates, it showed very few colonies and absorbance was relatively low compared to other temperatures. CFU cell

**Table 1.** Yeast cell counts after 48 and 72 h of incubation at different temperatures.

Temperature (°C)	CFU/ml		Log cell counts	
	48 h	72 h	48 h	72 h
30	1.33×10 <sup>6</sup>	2.53×10 <sup>5</sup>	6.12	5.40
32.5	1.67×10 <sup>6</sup>	2.07×10 <sup>5</sup>	6.22	5.31
37	9.8×10 <sup>5</sup>	1.8×10 <sup>5</sup>	5.99	5.25
40	6.7×10 <sup>5</sup>	1.7×10 <sup>5</sup>	5.82	5.23
45	7.0×10 <sup>4</sup>	6.0×10 <sup>4</sup>	4.84	4.77

**Table 2.** Yeast cell counts at different dextrose concentrations and at different time intervals after incubation for 48 h at 32.5°C.

Dextrose (concentration)	0 min (CFU/ml)	60 min (CFU/ml)	120 min (CFU/ml)	180 min (CFU/ml)	240 min (CFU/ml)	300 min (CFU/ml)
1x	3.9×10 <sup>5</sup>	7.7×10 <sup>5</sup>	4.6×10 <sup>6</sup>	3.36×10 <sup>6</sup>	2.96×10 <sup>6</sup>	2.54×10 <sup>5</sup>
3x	4.1×10 <sup>5</sup>	7.1×10 <sup>5</sup>	4.2×10 <sup>6</sup>	2.32×10 <sup>6</sup>	2.76×10 <sup>6</sup>	2.31×10 <sup>5</sup>
5x	4.5×10 <sup>5</sup>	6.5×10 <sup>5</sup>	1.28×10 <sup>6</sup>	3.72×10 <sup>6</sup>	2.60×10 <sup>6</sup>	1.23×10 <sup>5</sup>
7x	3.0×10 <sup>5</sup>	4.3×10 <sup>5</sup>	1.31×10 <sup>6</sup>	3.6×10 <sup>6</sup>	3.16×10 <sup>6</sup>	1.6×10 <sup>5</sup>
9x	2.7×10 <sup>5</sup>	2.1×10 <sup>5</sup>	1.25×10 <sup>6</sup>	2.3×10 <sup>6</sup>	2.84×10 <sup>6</sup>	1×10 <sup>5</sup>

counts after 72 h showed highest counts at 30°C and found to be lowest at 45°C (Table 1). Hence, 45°C is termed as the decisive growth temperature of this isolate (Figure 1b).

Diverse stages of osmotic pressure were promoted onward to attain the complete stress and heat response consequences of *C. shehatae* var *shehatae* upon critical temperature. A comparatively long lag phase was scrutinized in dextrose concentration from 120 min-240 min at optical density at 600 nm (Table 2). After 240 min, phase was observed to be going down (Figure 2a). On the other hand, sucrose concentration after 48 h 1X concentration had shown highest peak after 180 min of incubation (Table 3). Similar to dextrose concentration after 300 min, 9X sucrose concentration had shown the lowest peak (Figure 2b). About 4 log CFU/ml was eliminated in 7X and 9X dextrose concentrations as well as in sucrose concentration (Tables 2 and 3).

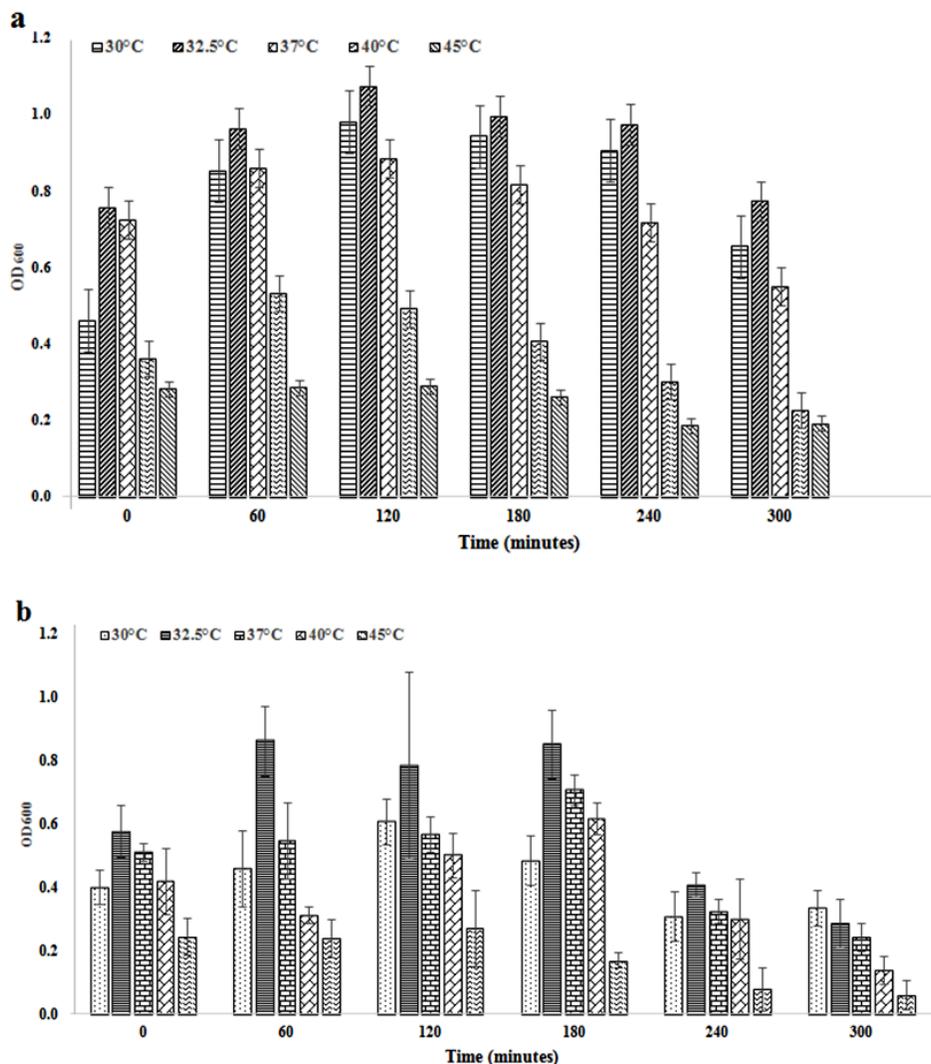
According to the previous studies, the yeast cells possibly showed an instantaneous growth arrest, while exposed to an increase in external osmolality. Interestingly in the morphological study, the budding cells start to become inactive at the possible temperature with 3X dextrose concentration. Cells also found to be thickened at 5X concentration and become dormant at 9X after 300 min of incubation (Figure 4).

## DISCUSSION

In previous studies, Munna et al. (2015) observed that yeast strain showed the optimum growth temperature at 32°C. However, other studies (Walsh and Martin, 1977) stated that between 25 and 35°C is the evincing point of

optimal growth temperature of the earlier *S. cerevisiae* (Tai et al., 2007; Salvadó et al., 2011). They also observed cell growth at 37 and 40°C, this was conceivably found due to the requirement of coping with the higher temperature for more time compared to the optimal growth conditions. Light microscope is used to observe the budding yeast. They also reported that, 40°C is the potential growth temperature of the yeast strain *S. cerevisiae*. On the other hand, a pointy fall was noticed in CFU and a comparatively minor depletion in the cell turbidity was observed at 44 and 45°C, respectively. Interestingly, at 45°C, cells were grown when the budding yeasts *S. cerevisiae* were grown to be inactive. Consistent with the effects of the growth estimation, all yeast cells completely lose their culture ability at 46°C as examined by the spot dilution tests. Hence, 45°C is termed as the critical growth temperature of this strain. Another study carried out by Moraitis and Curran (2004) reported, the reactive oxygen species influence in heat shock response as well as stress tolerances in the yeast *S. cerevisiae*. In the current study, *C. shehatae* var *shehatae* strain showed the same result under heat stress. Based on previous studies and after observing the growth level and cell turbidity at 44 and 45°C in this study, it can be concluded that when cells were grown at 45°C, the budding yeasts were noticed to become dormant. Thus, the critical growth temperature of *C. shehatae* var *shehatae* was established at 45°C.

Another study was conducted where physiological stresses have been employed to influence the levels of stress proteins and accrued polyols in yeast cells to find out the comparative involvements of these two factors to stress tolerance (Lewis et al., 1995). Previous investigations showed that in the presence of an



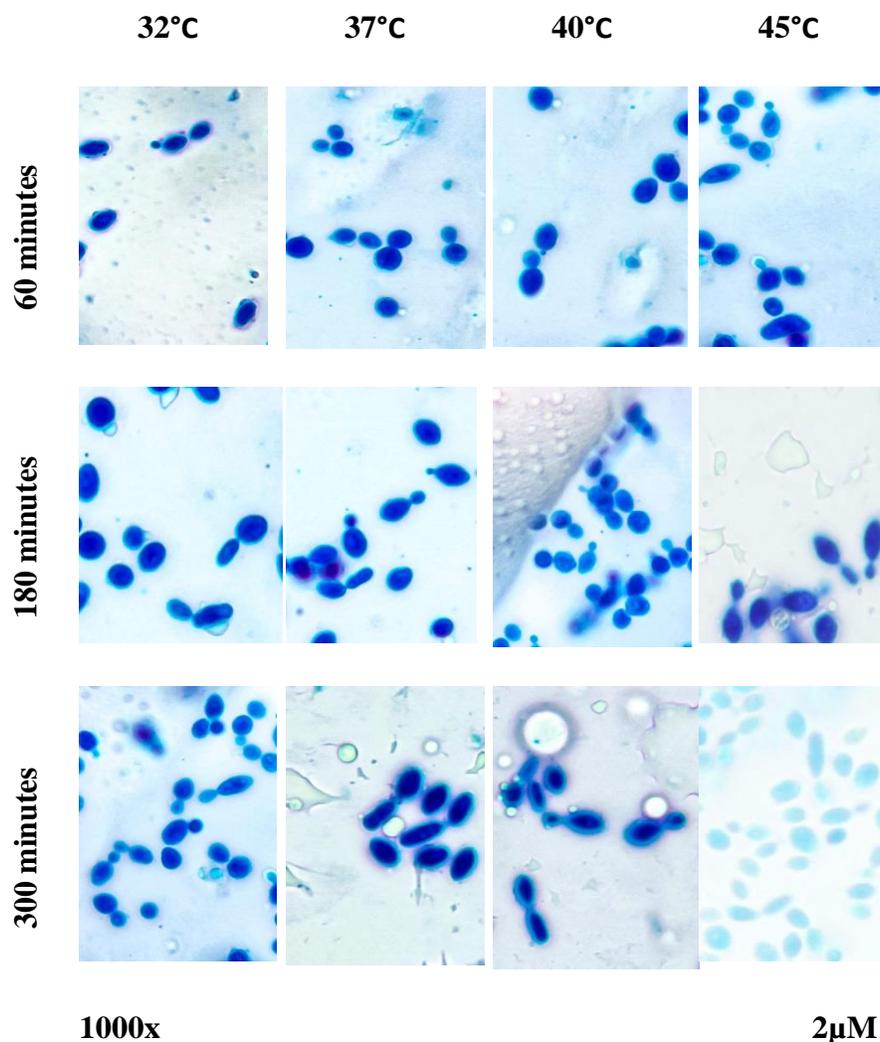
**Figure 2.** Complete stress (osmotic stress) response consequences of *C. shehatae* var *shehatae* upon critical temperature. Growth retardation of the strain at 32.5°C after 48 h with (a) dextrose concentration, (b) sucrose concentration.

**Table 3.** Yeast cell counts at different sucrose concentrations and at different time intervals after incubation for 48 h at 32.5°C.

Sucrose (Concentration)	0 min (CFU/ml)	60 min (CFU/ml)	120 min (CFU/ml)	180 min (CFU/ml)	240 min (CFU/ml)	300 min (CFU/ml)
1x	$3.1 \times 10^5$	$6.4 \times 10^5$	$1.76 \times 10^6$	$3.96 \times 10^6$	$1.67 \times 10^6$	$1.44 \times 10^5$
3x	$3.9 \times 10^5$	$6.2 \times 10^5$	$1.78 \times 10^6$	$4.36 \times 10^6$	$1.20 \times 10^6$	$2.61 \times 10^5$
5x	$4.1 \times 10^5$	$7.5 \times 10^5$	$1.20 \times 10^6$	$3.92 \times 10^6$	$1.80 \times 10^6$	$1.83 \times 10^5$
7x	$3.6 \times 10^5$	$5.8 \times 10^5$	$1.36 \times 10^6$	$3.55 \times 10^6$	$3.56 \times 10^6$	$1.92 \times 10^5$
9x	$2.3 \times 10^5$	$2.9 \times 10^5$	$2.00 \times 10^6$	$2.1 \times 10^6$	$1.84 \times 10^6$	$1.3 \times 10^5$

increased external osmolarity a sudden growth arrest may hit the yeast cells (Wuytswinkel et al., 2000). Further studies have shown that, to obtain the entire stress response, outcomes of *S. cerevisiae* upon decisive temperature and various levels of osmotic pressure were simulated onward (Munna et al., 2015). Same study was

conducted in this project where the yeast strain was further examined in order to find out the osmotic stress level. A lag phase was observed at 32.5°C in extended dextrose concentration. Approximately, 4 log CFU/ml was detected to be eliminated in 7X and 9X dextrose concentrations. Whereas the average was complemented



**Figure 3.** Morphological studies of *C. shehatae var shehatae* cells at different time intervals.

with very high (9X) dextrose concentration at 45°C cells and entirely lose their cultivability. Budding, a type of asexual reproduction cells, were quiescent at low concentrations and thickened at high concentrations. In this study, findings showed relatively long lag phase and the cells started to become thickened in 7X concentration and found to become dormant at 9X concentration. Consistent with the existing findings, Pratt et al. (2003) enlarged cell number of *S. cerevisiae* was also observed due to osmotic stress after 48 h of incubation. Hence, based on this study the cell volume was also found to expand after some time of incubation. However, an enormous amount of cells losing the cytoplasmic contents were scrutinized at 45°C with extremely elevated (9X) dextrose concentration. Consistently, in absence of osmotic imbalances, very few stressed cells (cells with no cytoplasmic contents) were detected at 45°C and completed growth suppression due to osmotic shock.

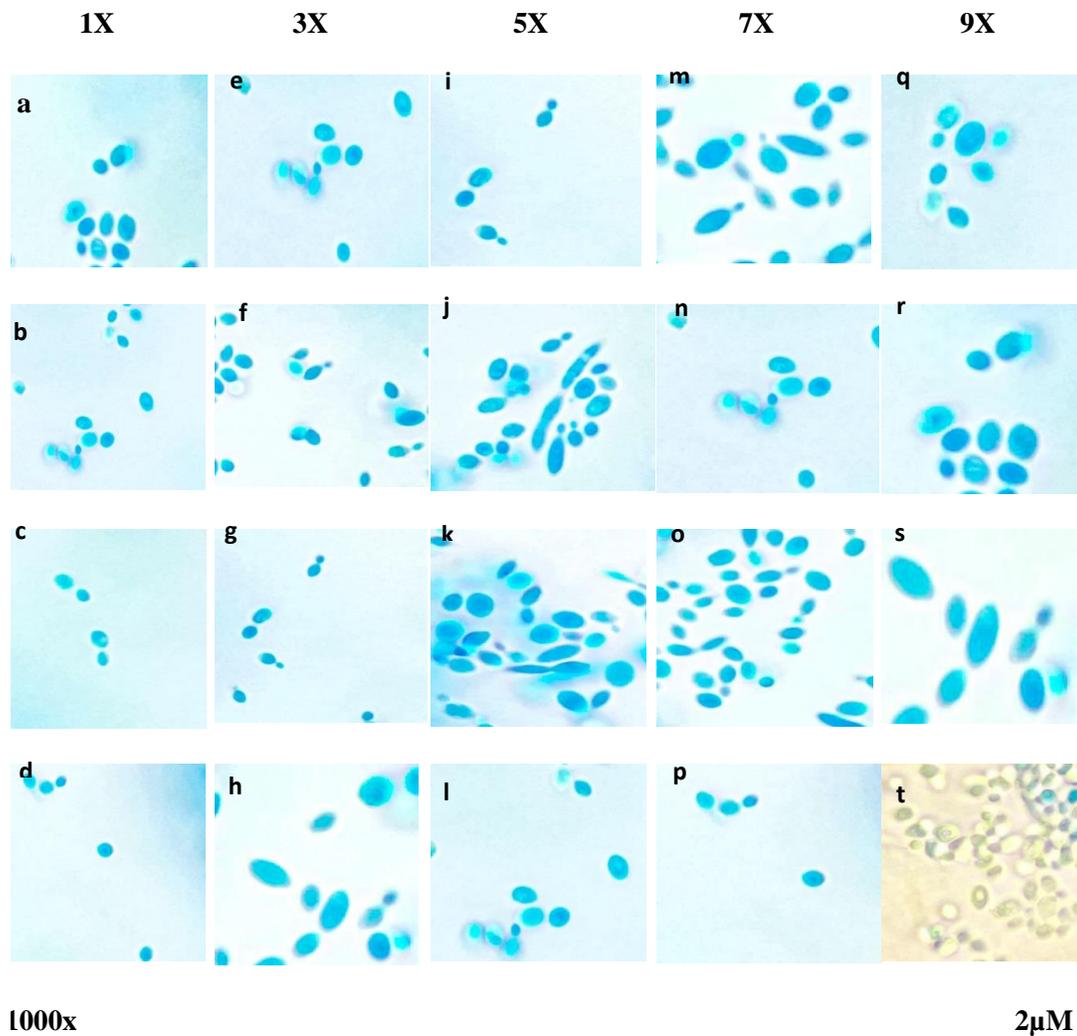
Another study conducted by Beney et al. (2001) provided the corroboration that viability of yeast cells decreases in the presence of increased osmotic pressure. The findings also demonstrated changes to the shape and characteristics of the yeast cells.

#### CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

#### ACKNOWLEDGEMENTS

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**Figure 4.** Morphological pattern at different dextrose concentrations: 1X, 3X, 5X, 7X, 9X-a, e, l, m, q (60 min); b, f, j, n, r (180 min); c, g, k, o, s (240 min); d, h, l, p, t (300 min).

and BCSIR authorities as well.

#### Data availability statement

The experimental data used to support the findings of this study are available from the corresponding author upon request.

#### Ethics statement

This article does not contain experiments using animals and does not contain human studies.

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