Genetic and Biochemical Analysis of the Reserve Carbohydrate Metabolism in *Candida albicans* and *Candida rugosa*

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Abstract

Stress response of an organism is important for its survival. In responses to abiotic stresses such as heat, oxidative stress and nutrient limitations yeast cells activates trehalose biosynthesis and accumulates trehalose as stress protectant agent. Glycogen is also accumulated within the yeast cells as reserve carbohydrates. Trehalose and glycogen metabolism are well characterized in *S. cerevisiae,* but there is a limited information on these carbohydrates in *candida species*. In this study, we have analyzed the stress-dependent accumulation patterns of trehalose and glycogen in *C. albicans* as a pathogenic yeast and *C. rugosa* as an industrial yeast. Our results indicate that there are clear differences in storage carbohydrate metabolism between these two yeasts species. Basal levels of glycogen in *C. rugosa*

is higher than the *C. albicans* glycogen content when these yeasts grown in normal conditions. However, when these yeasts subjected to stress inducing conditions, both trehalose and glycogen biosynthesis activated and rapidly accumulated within these yeast cells. Nonetheless, stress dependent activation of trehalose and glycogen biosynthesis in *C. rugosa* is much higher than the *C. albicans*. In addition, exposure of these yeast species to different abiotic stresses also resulted in activation of *TPS1* and *GSY1* gene transcription. The differences in trehalose biosynthesis and *TPS1* transcript levels indicates that reserve carbohydrate metabolism is differentially regulated in *C albicans* and *C. rugosa.*

Keywords: *Candida albicans*, *Candida rugosa*, Glycogen, Trehalose, Transcription

Introduction

Understanding the viability of commercial and pathogenic yeasts under nutrient, temperature, desiccation and oxidative stress is critical for the improvement of yeasts in biotechnological applications, and towards treatment of yeast infections. While coping with environmental stress, several factors such as heat shock proteins, lipid composition and storage carbohydrate content, play an important role in fermenting, non-fermenting and pathogenic yeast (Elbein et al., 2003 [1]; Simits et al., 2005 [6]). In particular, trehalose and glycogen, also known as reserve carbohydrates; have prominent protective roles versus environmental stresses (Roustan and Sablayrolles, 2004 [12]). The metabolism of trehalose and glycogen has been heavily investigated in several yeast species, particularly in *S. cerevisiae* (Silije et al., 1999 [7]; Vos et al., 2016 [26]).

In the yeast cell, glycogen is accumulated while glucose is still present in the medium and is only mobilized when all exogenous carbon sources have been exhausted. In addition to supplying carbon and energy to the cell under carbon-limited conditions, glycogen is also synthesized and degraded during the sporulation of diploid yeast cells. The key enzymes that is required for the glycogen synthesis in *S. cerevisiae* has been well documented (François and Parrou, 2001 [8]). Glycogen synthesis is catalyzed by glycogen synthase, which catalyze the formation of α-1-4 glyosidic bond and forms linear chain of glycogen molecule. Glycogen synthase encoded by *GSY1* gene in *S. cerevisiae. GSY1* has paralog gene *GSY2*. Glycogen branching enzyme encoded by *GLC3* gene, catalyzes the formation of α-1-6 glyosidic bond and generates side chains on linear arrays of glycogen chains in *S. cerevisiae*. Another enzyme; glycogen phosphorylase, encoded by *GPH1,* plays an important role in determining glycogen amounts in the cell (Tripodi et al., 2015 [4]; Wilson et al., 2010 [29]).

Trehalose is another important and multi-functional reserve carbohydrate for many yeasts. Its biosynthesis is activated by nutrient limitations and environmental stresses such as heat and oxidative stress (François and Parrou, 2001 [8]).Trehalose biosynthesis and degradation is tightly controlled by two opposing enzymes; trehalose synthase and trehalase, respectively. Trehalose is synthesized by the trehalose

phosphate synthase (TPS) enzyme complex in *S. cerevisiae* by consecutive two-step enzymatic reactions. The TPS enzyme complex comprises 4 different subunits; Tps1p, Tps2p, Tps3 and Tsl1p. The most critical subunit of TPS enzyme complex is Tps1p. Expression of *TPS1* is activated by general stress response factors, Msn2p/Msn4p through STRE sequence (5-CCCCT-3) which is present as multiple copies on the UAS regions of *TPS1* gene of *S. cerevisiae* (Cabib et al., 1958 [3]; Todorova et al., 2009 [27]). In addition to its stress protectant roles, trehalose controls glycolytic flux by restricting hexokinase activities via trehalose 6-phosphate – intermediary metabolites formed during trehalose biosynthesis (Gancedo and Flores, 2004 [2]). Recently, it has been shown that trehalose homeostasis is also controlled in a cell cycle dependent manner (Zhao et al., 2016 [15]). Recently, it has been reported that trehalose and glycogen contribute to stress tolerance in engineered yeast, that is yeast with more trehalose content showed higher fermentation capacity and significant stress tolerance (Wang et al., 2014 [20]). On the other hand, since there is no trehalose biosynthesis pathway in the mammalian cell, trehalose metabolism is therefore a promising target to design antifungal agents for pathogenic yeasts such as *C. albicans* (Argüelles, 2017 [10]; Rayne et al., 2017 [25]).

Although, many research reports are available on the regulation of trehalose and glycogen metabolism, and their functions in the baker's yeast, there is a limited information on trehalose and glycogen homeostasis in *C. albicans* and *Candida rugosa* (Elbein et al., 2003 [1]; Parrou et al., 1997 [11]; Borocco et al., 2003 [22]).

On the basis of current studies, we have investigated the effects of different stress conditions on the trehalose and glycogen biosynthesis in *C. albicans* and *C. rugosa*. Moreover, the expression profiles of *TPS1* and *GSY1* genes were also analyzed in *C. albicans* and *C. rugosa*. Our results indicated that trehalose and glycogen metabolism is induced by abiotic stresses in these two *Candida* species. Nonetheless, it is clear that trehalose and glycogen homeostasis shows differences in these two yeasts species. It appears that *C. rugosa* has higher levels of trehalose and glycogen than *C. albicans* under normal or stress inducing conditions. Expression levels of *TPS1* and *GSY1* genes also shows differences between these two yeasts species.

Material and methods
Yeast strains and growth conditions
The yeast strains used in this study and their sources are; *Candida albicans* (ATCC14053), *Candida rugosa* (NRRL-Y-95) strains were cultivated in YPD (1% yeast extract, 2% peptone, 2% dextrose) medium for routine uses and stored at 4 °C during experiments. To determine trehalose and glycogen contents, yeast strains were pre-cultured in 10 ml YPD medium overnight at 30 °C in an orbital shaker (140 rev/min). These saturated pre-cultures were used to inoculate the fresh cultures. The initial cell densities of fresh yeast cultures were adjusted to OD_{600}: 0.1-0.2 and grown to the logarithmic stage at standard growth conditions (at 30 °C, 140 rev/min in an incubator shaker). At the logarithmic stage, stress inducing agents were applied to the growth medium as explained in the sections below.

Induction of stress conditions

In order to analyze the effects of glucose limitation on the trehalose and glycogen amounts, yeast species were inoculated in 10 ml of YP medium supplemented with 2% acetic acid and cultivated overnight. Fresh medium with same ingredients were inoculated OD_{600}=0.1 final concentration and cells were grown up to the logarithmic stage.

For heat shock, yeast strains were grown at 25 $^{\circ}$C up to pre-log stage first, then rapidly shifted to a 39 $^{\circ}$C incubator shaker and grown for 2 h. To analyze the effects of oxidative stress and metal ions, yeast cells were grown in 10 ml of YPD medium to pre-log stage in standard growth conditions, then stress inducing agents (40 μM menadion, 0.1mM cobalt, iron, and cadmium for each) were added to yeast cultures, and further incubated for 2 hours (Todorova et al., 2009 [27]). At the end of incubation periods, yeast cells were harvested to determine trehalose and glycogen amounts as described (Parrou et al., 1997 [11]). Wet mass of yeast pellets was determined to normalize trehalose and glycogen amounts of yeast species.

Enzymatic assay

Trehalose and glycogen contents in the yeasts samples were determined enzymatically as described by Parrou et al., (1997) [11]. Briefly, trehalose and glycogen moieties of yeast cells were hydrolyzed with 3 mU of trehalase (Sigma T8778) and 100 μg α-amyloglucosidase (Sigma a-7420) as described (Parrou et al., 1997 [11]). The amounts of glucose released from enzymatic digestion of trehalose and glycogen were determined enzymatically in duplicates using glucose oxidase-peroxidase glucose determination kit (GOD-POD) (Spinreact) as described by the manufacturer (Parrou et al., 1997 [1]]). Concentrations of trehalose were expressed as mg glucose equivalent to per gram of wet mass (mg/g) of the yeast cells (Türkel, 2006 [23]). Yeast cultures were grown in duplicates in all experiments and the experiments were repeated twice. Hence glycogen or trehalose contents given in the tables are the average values of the eight independent measurements.

Analysis of tps1 and gsy1 mRNA levels by RT-PCR

Total RNA was extracted from 10 mL yeast cultures that were grown under normal or stress conditions using RNeasy mini kit (Qiagene). Magna Lyser (Roche) ceramic beads were used to break down the cell wall. Extracted total RNA was treated with 3 units of DNase (Promega RQ1 RNase-free DNase). cDNAs were synthesized from 1 μg RNA with high fidelity cDNA synthesis kit (Roche). Since there is no record of sequences of the *tps1* gene in *C. rugosa*, degenerate primers were designed for *C. rugosa* aligning *tps1 and gsy1* gene sequences from *C. albicans*, *Candida dubliensis*, *Candida tropicalis* and *Candida glabrata* species. Sequences of *tps1* and gsy1 primers used in RT-PCR are given in **Table I**. Reaction mixture for RT-PCR was prepared with fast start essential DNA green master mix (Roche). Cycling parameters were 95 $^{\circ}$C for 10 min; 45 cycles (95°C for 20 s 55°C

for 20 s, 95 ºC 30 s) and finally 65 ºC for 60 s 95 ºC 1 s 40 ºC 30 s. Each sample was tested in triplicates. Fold changes were calculated using the comparative ΔΔCt method using *ACT1* ORF as a reference.

Tablo 1. Primers sequences for TPS1 and GSY1 transcripts for real-time analyses

Primer	Primer Sequence
Candida ACT1 F	5' ATGTGTAAAGCCGGTTTTGCCG 3'
Candida ACT1 R	5' CCATATCGTCCCAGTTGGAAAC 3'
Candida TPS1 F	5' TGGCCACTTTTCCATTATCA 3'
Candida TPS R	5' CATTAAATGRTAATCATGAACCCA 3'
Candida GSY F	5' CTTGGGGTTACACKCCWGC 3'
Candida GSY R	5' TTGRTTGATTCTTTGTCTTCTGG 3'

Results

**Trehalose and glycogen accumulation under carbon restriction**

Acetic acid can be metabolized by yeast via acetate once it is transported into yeast cells. Acetic acid also activates apoptosis in yeasts (Ludovico et al., 2001 [19]). To see the effects of acetic acid stress on the reserve carbohydrate metabolism, *Candida* species grown in acetic acid medium and then reserve carbohydrate levels were determined. It is quite surprising that *C. albicans* and *C. rugosa* cells are not able to accumulate any trehalose in response to acetic acid. We could not detect any measurable trehalose in *C albicans* and *C rugosa* in *Candida* species incubated in acetic acid medium **(Figure 1).** Contrary to trehalose biosynthesis, acetic acid induced glycogen biosynthesis more than 2-fold in *C. albicans*. But same was not true for the glycogen levels in *C rugosa*. Glycogen level decreased nearly 14-fold (from 8.04 mg to 0.59 mg) in *C. rugosa* when exposed to acetic acid stress **(Figure 1).**
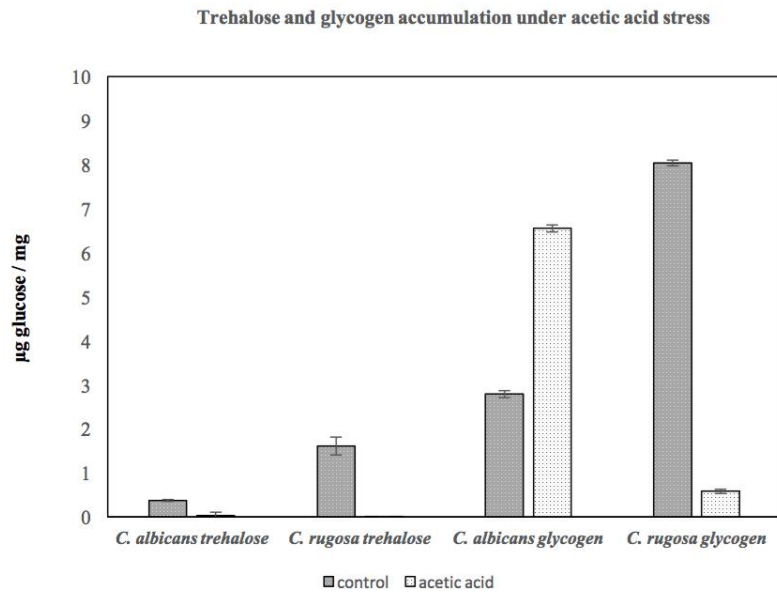
Figure 1. Trehalose and glycogen content of *C. albicans* and *C. rugosa* under acetic acid stress. Cultures were grown in YP + acetic acid medium and cultures were exposed to stress up to the logarithmic stage. Error bars indicate the standard deviations.

Trehalose and glycogen accumulation in *Candida* species under heat stress

It is known that the biosynthesis of reserve carbohydrates is activated when the actively growing yeast cells are expose to stress inducing conditions. Temperature stress is an effective inducer of the trehalose biosynthesis in *S. cerevisiae*. To see the effects of temperature stress on the trehalose and glycogen metabolism, *Candida* species used in this study were pre-grown at 25 $^{\circ}$C to early log stage and then shifted to 39 $^{\circ}$C for at least 2 hours. Temperature stress induced accumulation of both storage carbon sources in both yeasts without exception. The increase in trehalose amount was significant both in *C. albicans* (9.59-fold) and in *C. rugosa* (3.74-fold) under heat shock **(Figure 2)**. But, glycogen content of these yeasts did not show significant changes in response to heat stress. Glycogen amount of *C. albicans* remained at same levels in heat stressed yeast cells. Nonetheless glycogen amount in *C. rugosa* cells increased about 1,59-fold when exposed to heat stress for 4 hours **(Figure 2)**.
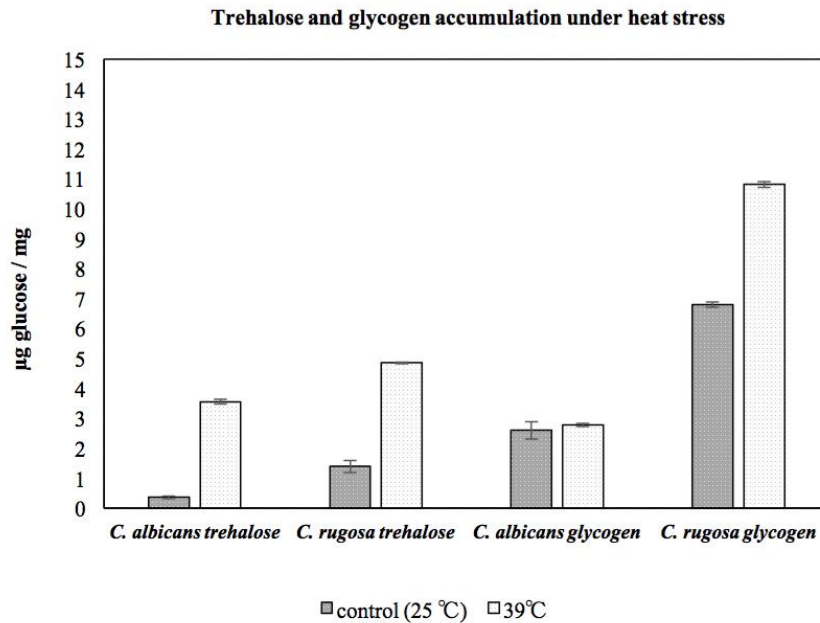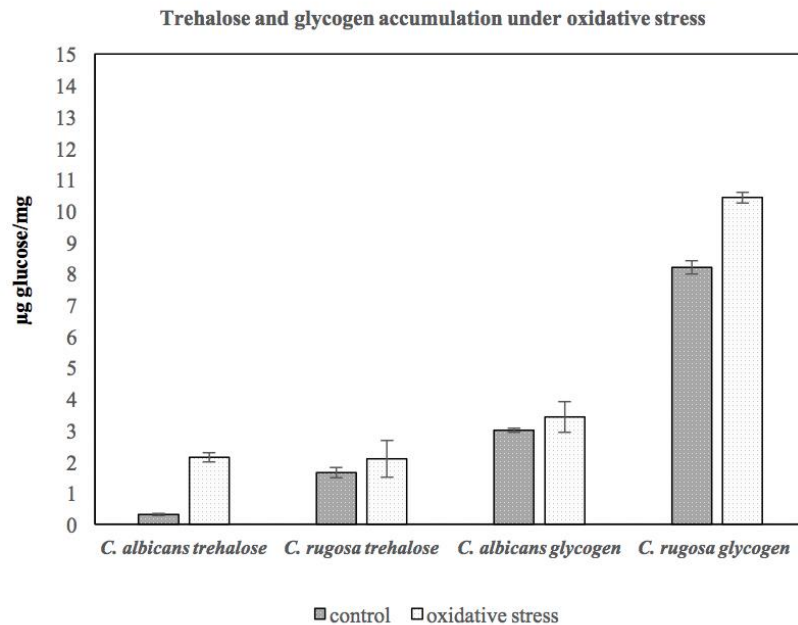
Figure 2. Trehalose and glycogen content of *C. albicans* and *C. rugosa* under heat shock stress. Cells were incubated at 25 °C up to the early logarithmic stage first, and then to apply heat stress, cells were transferred to 39 °C. After 4 hours, cells were collected and trehalose and glycogen amounts were determined. Error bars indicate the standard deviations

Trehalose and glycogen accumulation under oxidative stress in *C. albicans* and *C. rugosa*

Oxidative stress generated by reactive oxygen species is another inducer of the trehalose biosynthesis through the general stress response pathway mediated by STRE in *S. cerevisiae*. To generate oxidative stress in the *Candida* cells, yeast cultures were exposed to 40 mM menadion for 2 h, then trehalose and glycogen contents of the yeast cells were determined. As in the heat stress, oxidative stress also induced trehalose and glycogen accumulation in both yeasts. Trehalose content of *C. albicans* elevated from 0.32 mg/g wet mass (basal level) to 2.14 mg/g wet mass (6.79-fold increase) in response to oxidative stress **(Figure 3)**. However, it seems that oxidative stress is less effective on the activation of trehalose biosynthesis in *C. rugosa*. Trehalose amount of *C. rugosa* was only 1.09-fold higher than the basal level (unstressed) *C. rugosa* cells.

Unexpectedly, oxidative stress did not activate the glycogen accumulation *C. albicans* and *C. rugosa*. The glycogen amount of *C. albicans* remained at basal levels in *C. albicans* in menadion exposed cells **(Figure 3)**. The slight increase in the glycogen amount (from 8.20 mg/ to 10.43 mg) was determined in response to menadion generated oxidative stress in *C. rugosa* cells **(Figure 3).**

Figure 3. Trehalose and glycogen content of *C. albicans* and *C. rugosa* under oxidative stress. Cells were incubated with 40 µM menadion to create oxidative stress. Cells were grown up to the logarithmic stage first, collected and trehalose and glycogen amounts were determined. Error bars indicate the standard deviations

Metal ion stress affects trehalose and glycogen accumulation in *Candida* species

In order to test the effects of toxic metal ions on the trehalose and glycogen accumulation in *C. albicans* and *C. rugosa*, yeasts were treated with different metal ions (cobalt, iron, cadmium) at the logarithmic stage. All three metal ions activated trehalose biosynthesis in *C. albicans*. Especially cadmium and iron activated trehalose accumulation up to 7-fold in *C. albicans* (**Figure 4**). The effects of cobalt stress on the trehalose accumulation was less than the cadmium in *C. albicans*. Metal ions exerted differential effects on the trehalose metabolism in *C. rugosa*.

Presence of cobalt ions in the growth medium resulted with 4.6-fold decrease (from 1.61 mg to 0.35 mg) in the trehalose level of *C. rugosa* (**Figure 4**). Moreover, cadmium ions have no effects on the trehalose accumulation in *C. rugosa*. Trehalose amount remained at same levels (1.72 mg) in cadmium exposed *C. rugosa* cells. On the other hands, iron ions activated trehalose accumulation in *C. rugosa* less than 2-fold (from 1.61 mg to 3.05 mg) (**Figure 4**). The effects of these metal ions on the glycogen accumulation were a lot less than the trehalose accumula-

tion. Cobalt ions did not have any effect on glycogen levels in *C. albicans* and *C. rugosa* (**Figure 4**). Iron and cadmium activated glycogen less than 2-fold in *C. albicans*. On the other hand, cobalt ion stress lead to slight decrease in *C. rugosa*, while cadmium stress had no effects. Iron ion stress result with slight increase in glycogen content (from 8.2 mg to 12.95 mg) of *C. rugosa* (**Figure 4**).
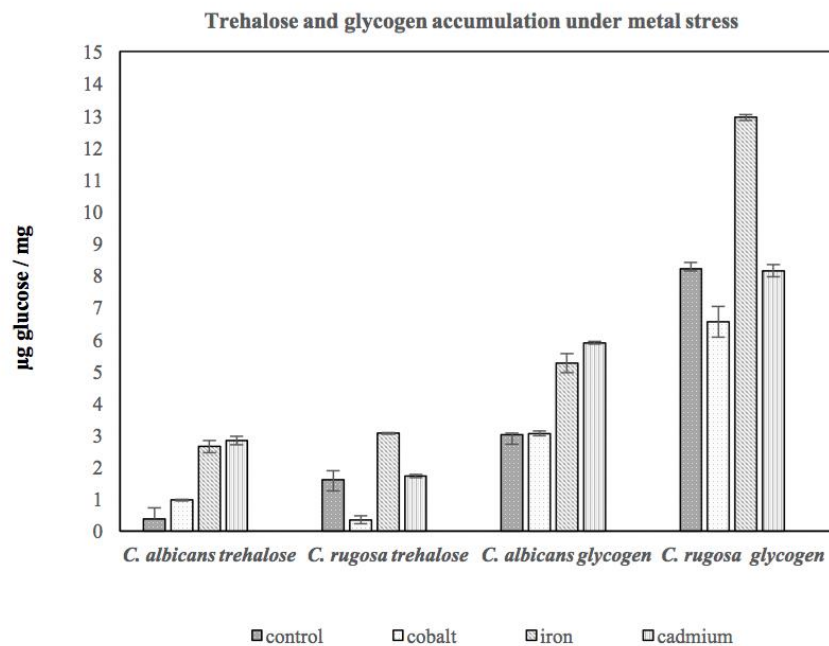

Figure 4. Trehalose and glycogen content of *C. albicans* and *C. rugosa* under metal stress. Cells were incubated with 0.1 mM metals seperately to create oxidative stress. Celle were grown up to the logarithmic stage first and then collected and trehalose and glycogen amounts were determined. Error bars indicate the standard deviation

Transcription profiles of TPS1 and GSY1 gene under stress conditions

TPS1 and *GSY1* are two genes that encodes key enzymes in the trehalose and glycogen biosynthesis in *S. cerevisiae*. It is known that the transcription of *TPS1* is activated in response to unfavorable growth conditions in *S. cerevisiae* [10]. After analyzing the accumulation patterns of the reserve carbohydrate levels in response to various environmental stress conditions in *Candida* species, we wanted to analyze the mRNA levels of *TPS1* and *GSY1* in *Candida* species. We have shown that the heat stress activated trehalose biosynthesis and result with high level of trehalose both in *C. albicans* and also in *C. rugosa* (**Figure 2**). It is surprising that

the heat stress did not activates the transcription of TPS1 in *C. albicans* and *C. rugosa* **(Figure 5)**. Moreover, *TPS1* expression level slightly decreased in cadmium exposed yeast cells. We did not see significant level of transcriptional activation of *TPS1* gene neither in *C. albicans* nor in *C. rugosa* when exposed to all stress conditions except acetic acid stress **(Figure 5)**. Transcription of *TPS1* activated nearly 4-fold only in acetic acid exposed *C. albicans* cells.
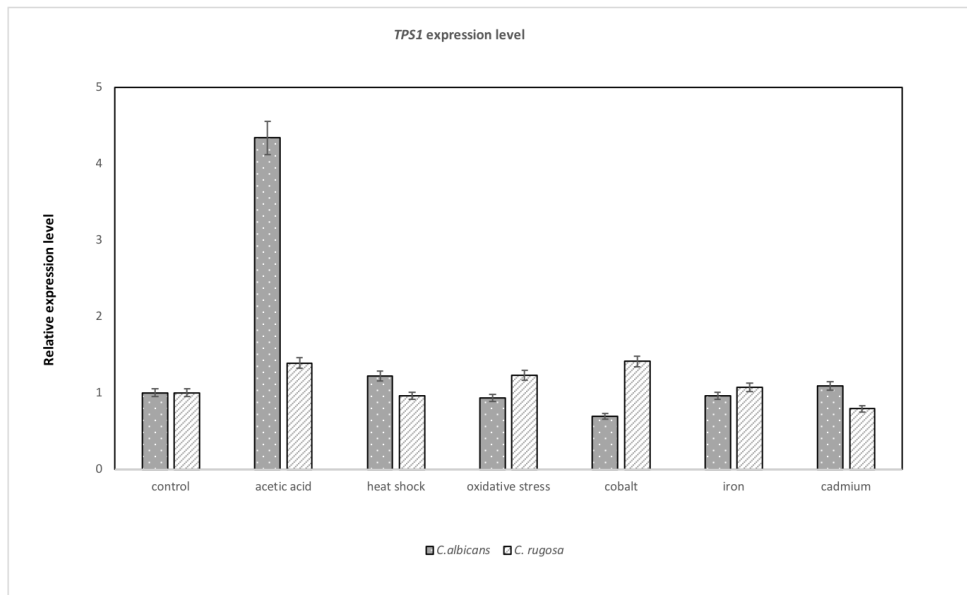
Figure 5. Expression levels of *TPS1* gene in *C. albicans* and *C. rugosa* under different stress conditions. Fold changes were calculated using the comparative ΔΔCt method using *ACT1* ORF as a reference

Unlike *TPS1* expression level, *GSY1* expression level activated at high levels (5 to at least 70-fold) in *C. albicans*. The highest-level activation of *GSY1* gene transcription determined in iron and cadmium exposed *C. albicans* cells **(Figure 6)**. On the other hand, *GSY1* gene transcript levels remained at nearly basal level in *C. rugosa* exposed to various stress inducing agents.
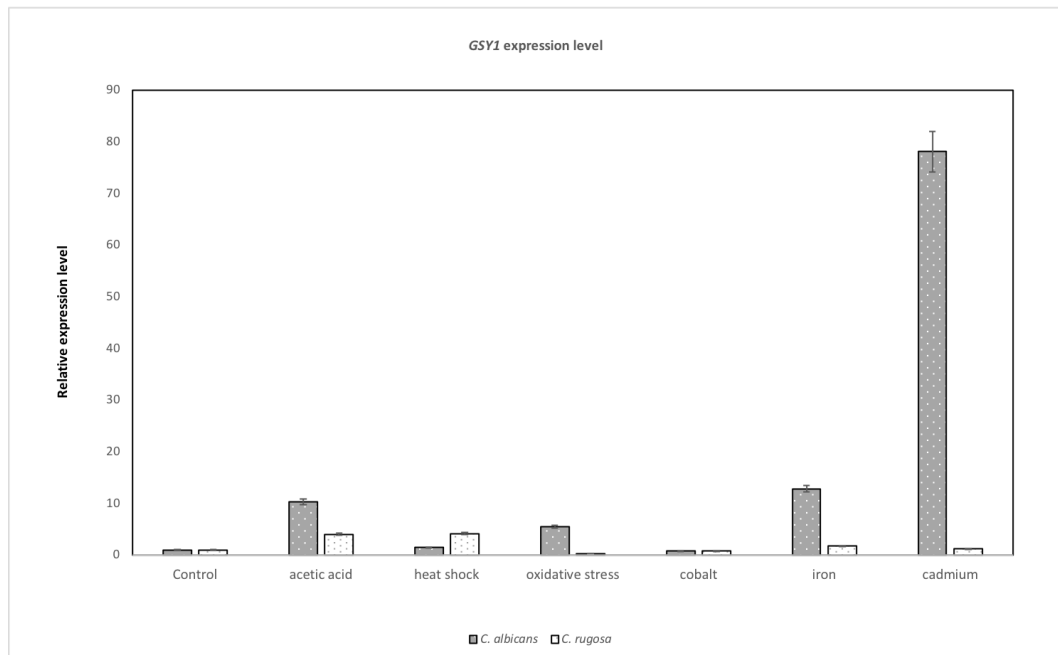
Figure 6. Expression levels of *GSY1* gene in *C. albicans* and in *C. rugosa* under different stress conditions. Fold changes were calculated using the comparative ΔΔCt method using *ACT1* ORF as a reference

Discussion

There is a strict correlation between reserve carbohydrates and stress response in yeasts. Trehalose controls glucose flux and prevents protein denaturation in response to various stress conditions. However, cytoplasmic functions of trehalose depends not only on different stress conditions, but also on the metabolism of the yeast cells (Lillie and Pringle, 1980 [24]; Shi et al., 2010 [14]). Although different yeast species have developed different stress responses against various stress conditions, current stress-response research is focused on either some specific stress conditions or on some specific yeasts. In this research, we have investigated the biosynthesis and accumulations of trehalose and glycogen in response stress conditions in non-conventional yeasts *C. albicans* and *C. rugosa*. We have shown that both trehalose and glycogen accumulation patterns differs depending on stress conditions in these two *Candida* species. Heat stress activated trehalose biosynthesis and accumulations in both yeasts. However, it is clearly noticeable that *C. rugosa* has much higher levels of (2- to 3-folds) trehalose and glycogen then *C. albicans* under normal growth conditions. Interestingly, heat stress did not result with glycogen accumulation in *C albicans*. Elbein et. al. (2003) [1] reported that *C. albicans* lacks general stress response pathway. They suggested that *C. albicans* do not accumulate trehalose and glycogen in response to mild stress conditions (such as 37 C, 0.4 mM H_2O_2). However, in their experimental set up, they apply low levels of stress only for 30 to 60 min, as oppose to our stress inducing

conditions. Our results indicated that *C. albicans* and *C. rugosa* accumulates trehalose in response to heat and oxidative stresses. Lack of glycogen accumulation in heat stressed *C. albicans* might result from the rapid turnover of glycogen in this yeast. It was remarkable how *C. rugosa* exhibited high trehalose and glycogen accumulation even in non-stress conditions. *C. rugosa* attracts attention as an industrial yeast with lipase biosynthesis and a possible pathogenic yeast.

Our results indicated that acetic acid stress effected glycogen accumulation in *Candida albicans*. But the other yeast C. *rugosa* did not accumulate trehalose or glycogen under acetic acid stress. These results are not consistent with the results revealed by early studies that *S. cerevisiae* accumulated trehalose rapidly under weak acid stress created with sorbic acid (Cheng et al., 1999 [13]). On the other hand, parallel to our results, Yoshiyama et al. (2015) [30] reported that acetic acid challenge did not cause the rapid accumulation of trehalose in the exponential phase of cell growth, but they maintained that the accumulation of trehalose helps cells during fermentation under acetic acid stress. The type of organic acid can also have an effect on storage carbohydrate metabolism. When we analyzed the *TPS1* and *GSY1* expression level of both organisms, we observed that both gene expression levels were increased in both yeasts. In parallel to the biochemical results; the increase in *GSY1* level was much higher, i.e. 10-fold greater than the other result. The 4-fold increase was not reflected in the product amount, but the 10-fold increase was reflected in the glycogen accumulation.

External conditions; heat shock and oxidative stress are important especially for industrial yeasts. Besides the physiological role, trehalose could also find industrial applications in creating engineered strains able to withstand environmental stress. When trehalose is discussed, the most important stress condition considered is heat shock. Heat shock response (HSR) of *S. cerevisiae* is activated at temperatures greater than 37 °C. *S. cerevisiae* is able to cope with high temperatures of up to 42 °C (Argüelles, 1997) [9]. *C. albicans* has a commensal relationship with warm-blooded organisms and thus would be expected to live in a relatively stable environment in terms of temperature. It is for this reason that most of the studies are interested in the heat shock response for *C. albicans*. Alvarez-Peral (2002) [5] elucidated that heat shock stress did not affect the viability of the wild type and *tps1/tps1* mutant. It was emphasized that pre-incubation led to the accumulation of trehalose more than the cell exposed to heat shock in the long term. Supporting the data, our biochemical results showed that heat shock caused the accumulation of trehalose and glycogen. Trehalose accumulation of *Candida albicans* was almost 10-fold compared to the control but *TPS1* expression level was 1.2-fold. Under heat shock stress, the stress protectant role of trehalose emerges. Our results showed that, *TPS1* level of both organisms did not increase significantly. Previous studies indicated that Δtps1 mutant showed hypersensitivity to various stress. On the contrary, Petitjean and et al. (2015) [16] emphasized that under heat shock stress and oxidative stress, trehalose does not have protective role; the main role is played by trehalose-6P- synthase (Tps1) (Petitjean et al., 2015) [16]. We can conclude that the level of trehalose increased under heat shock, but this is not

directly related to *TPS1*. Glycogen level of both organisms was not increased significantly under heat shock and *GSY1* transcript level of *C. albicans and C. rugosa* increased 1.45-fold and 4.17-fold, respectively. Oxidative stress is a main stress factor for pathogenic yeasts because the destructive response of immune cells against pathogenic microbes are based on reactive oxygen species. Trehalose deficient mutants of *S. cerevisiae* and *C. albicans* showed sensitivity under hydrogen peroxide stress (Zaragoza et al., 2003 [17]). In this regard, our results agree with previous studies. Sanchez- Fresneda et al. (2013) [21] showed trehalose accumulated under oxidative stress in *Candida albicans,* as well. Response of *C. albicans* to the oxidative stress was higher than *C. rugosa*. Pathogen organisms cope with the ROS during infection (Gonzales-Parraga et al., 2010) [18]. Oxidative stress response could be dependent on the virulence factors. Studying of storage carbohydrate metabolism raises new and interesting research questions. Reaction of yeasts to metal ions depends on the uptake of ions and heavy metal exposure triggers the production of reactive oxygen species. Therefore, the subsequent response to the metal ions should be similar to the oxidative stress response. Indeed, the accumulation in both yeasts was not surprising because similar results were reported in oxidative stress, with the quantity of trehalose in *C. albicans* showing the highest increase. The most significant increase in *gsy1* expression level was observed under cadmium stress to levels as high as 78-fold. Early studies on glycogen metabolism mostly focused on GSY2 and not GSY1, since glycogen levels of *GSY2* mutant cells decreased dramatically. Data obtained here demonstrates that GSY1 expression level is also affected by different stress conditions. Unnikrishnan et al., (2003) [28] characterized the promotor of GSY1. As a future perspective, our goal will be to evaluate the promoter of GSY1 as a stress inducible promoter with data obtained from this study.

Glycogen is described as a storage carbohydrate and trehalose mostly described as a stress protectant. The accumulation time of trehalose and glycogen differs in the cell. Early studies showed that glycogen accumulates while nutrients are still available but trehalose accumulation occurs under glucose depletion and cells prefer to use glycogen first at the stationary phase. From this perspective, trehalose and glycogen metabolism is quite complex. This study focused mainly on the storage carbohydrate metabolism of two different yeasts which belong to the same species, but which have different significance.

Response of the cell against heat shock, oxidative stress, iron stress and cadmium stress were parallel with increasing amounts trehalose and glycogen in the cell. Response of the cells to starvation was different but on the other hand, we could not see very significant changes in the storage carbohydrate metabolism. The most interesting result was that even during non-stress conditions, trehalose (1.62 μg glucose / mg) and glycogen (8.04 μg glucose / mg) amounts in *C. rugosa* were higher than the storage carbohydrates amounts in *C. albicans* (**Figure 3**) which could make *C. rugosa* more robust for industrial usage.

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