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DEVELOPMENT OF SOME TOLERANT YEAST (*SACCHAROMYCES CEREVISIAE*) STRAINS TO HEAT AND SALT STRESSES

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ABSTRACT

All living organisms are subjected to changing in conservational conditions, to which they must adapt to**.** Stress is defined as a threat refers to the physiological balance of systems critical to survival organisms. Five yeast strains (*Saccharomyces cerevisiae*) were subjected to different adverse environmental situations, such as thermal, osmotic and oxidative (salt) stresses. The objective of this work was to detect the most tolerant yeast strains under salt or heat stresses. Five yeast strains were exposed in a first experiment to heat stress at 20°C or 40°C beside to the control at 30°C to detect the more tolerant strain. The same yeast strains were subjected in a second experiment to two different concentrations of salt stress (NaCl); 0.5 or 1.0 M, separately, for two days (at 30°C as normal temperature for growth), other strains were exposed to 0.5 M concentration of NaCl for 24 hours, then 1 M for another 24 hours. For the heat stress results, strain S4 was more tolerant at 40^{o}Cwith insignificant difference compared to the control (30^{o}C), while it showed significant difference at 20°C. Strain S5 also was more tolerant at 20^{o}C with insignificant difference compared to the control. For salt treatment, the only insignificant value was for strain S3at 0.5M NaCl compared with the control.

Key words: Yeast, *Saccharomyces cerevisiae,* Stress response, Heat stress, *Salt stress*.

INTRODUCTION

Yeast (*Saccharomyces cerevisiae*) strains have been extensively studied in recent years for fuel ethanol production, in which yeast cells are exposed to various stresses such as high temperature, ethanol inhibition, and osmotic pressure from product and substrate sugars as well as the inhibitory substances released from the pre-treatment of biomass. An in-depth understanding of the mechanism of yeast stress tolerance contributes to breeding and gave more robust strains for ethanol production, especially under very high gravity conditions (**Zhao and Bai, 2009).** Organisms are continually challenged by changing environments. They have evolved physiological adaptations to cope with such change. These mechanisms include genome-scale gene expression changes on short, physiological time scales. Such changes have been most thoroughly studied in stressful environments, such as heat, cold, osmotic stress, salt, drought, gene-toxic stress, ultraviolet light, oxidative stress, wounding, and pathogen infection **(Gasch et al 2000 and Jozefczuk et al 2010).** Two types of stress response have been observed, one corresponding to a specific response involving genes with functions related to the tested stress, and the other to a more general response observed regardless of the type of stress involved **(Brion, 2016).** The objective of this study was to detect the most tolerant yeast strains exposed to heat or salt stress.

MATERIALS AND METHODS

Materials

1. Yeast strains

Five yeast strains were used in this study, their names, codes and sources are shown in **Table 1.**

Table 1. Names, codes and sources of the five yeast strains (*Saccharomyces cerevisiae*)

Yeast strains name	Code	Source
UQM-49	S1	Microbial Resources center (Cairo MIRCEN)
NRRLY-17008	S2	Microbial Resources center (Cairo MIRCEN)
LBC-1208	S3	Microbial Resources center (Cairo MIRCEN)
LBC-254	S4	Microbial Resources center (Cairo MIRCEN)
ATCC-58523	S5	Dr. Mohamed Adel, Food Science Dept., Fac. of Agriculture, Ain Shams University.

2. YPG medium

Yeast peptone glucose (YPG) medium was used according to **Curran and Bugeja (2006)**. The standard YPG medium consisting of 1% yeast extract, 2% peptone, 2% glucose and 2% agar routinely used for maintenance and preparation of cultures for yeast experiments.

Methods

1. Yeast stress experiments

1.1. Heat stress

Heat stress was performed at 20^{o}C and 40^{o}Cin YPG broth culture for 2 days compared with the control at 30^{o}C.The strains were grown over night at 30^{o}C, then in the next day at 20^{o}C overnight again and the strains were grown overnight at 30^{o}C and the next day at 40^{o}C overnight using **Gasch (2000)** method.

1.2. Salt stress

Salt stress was applied using two concentrations; (0.5M NaCl; 2.9 gm up to 100 ml YPG medium, 1M NaCl; 5.8 gm up to 100 ml YPG medium). In the first experiment, the five yeast strains were grown on YPG medium, with 0.5 M or 1 M NaCl concentrations, separately, for 2 days at 30°C (normal growth temperature).

In another experiment, the yeast strains were put under the influence of 0.5 M of NaCl for 24 hours, then in the next day, they were grown on 1 M NaCl for another 24 hours at 30°Caccording to **Logothetis et al (2007).**

2. Dilution plating

This procedure was used to identify the number of viable micro-organisms (colonies) in a fixed amount of a liquid medium. It can also be fairly easily modified to give results with solid medium according to **Jorgensen and Turnidge (2015)** method.

To perform serial dilutions, a small amount of YPG (yeast extract, peptone, glucose and agar) medium was transferred into a new container to dilute the original solution. The diluted sample was then used as the base solution to make an additional dilution. This was carried out several times in a range of concentrations. The initial concentration with target range needed for a given assay determined the size and number of dilution steps required. Often, serial dilutions were performed; they were described as ratios of the original and final concentrations. Mixing 100 µl of a stock solution with 900 µl of water makes a 1:10 dilution. The final volume of the diluted sample was 1000 µl (1 mL) and the concentration was 1/10 that of the original solution. This was commonly referred to as a 10x dilution. Five µl was obtained from dilutions and put in Petri dishes; each experiment was made in 3 replicates for every strain.

3- Statistical analysis

The obtained data were statistically analyzed by using SPSS version 19.0 **(Arbuckle, 2010)** to compare of the mean duration of stress using analysis of variance (ANOVA) between the various treatment groups. This was performed using Dunnett test **(Dunnett,1964).**

RESULTS AND DISCUSSION

The results of heat and salt experiments appear in **Table (2) and Figure (1).** The five yeast strain**s** showed different responses under heat or salt stresses compared with the control. Three

replicates for each strain were applied for each treatment. Means of these replicates were calculated and differences were measured statistically.

1- Effect of stresses on Yeast strains

1- Heat stress

Heat stress results in **Table (2) and Figure (1)** showed that strain S4 was more tolerant at 40^{o}C with insignificant difference compared to the control (30^{o}C), while, the same strain gave significant difference than the control at 20°C and confirmed that it was more tolerant at the two heat stress experiments. Strain S5 also was more tolerant at 20^{o}C with insignificant difference compared to the control. The strains exposed to a temperature at 20°C or 40°C had a difference between the control (30°C) were previously detected by **Holubarova et al (2000)** who exposed yeast cells at 41°C and found intolerance of yeast cells.

2- Salt stress

For salt experiments, **(Table 2 and Figure1),** four strains showed decrease in number of colonies with significant differences under all salt treatments when compared with the control. Strain S3 gave the only insignificant number of colonies (19 colony) when grown in medium with 0.5M NaCl separately compared to the control (24 colony). The general mean of this work was to gain further understand of yeast fermentation performance as number of colonies under salt stress conditions, especially, work was focused on the evaluation of NaCl induced salt stress responses on industrial yeast strains (*Saccharomyces cerevisiae*).

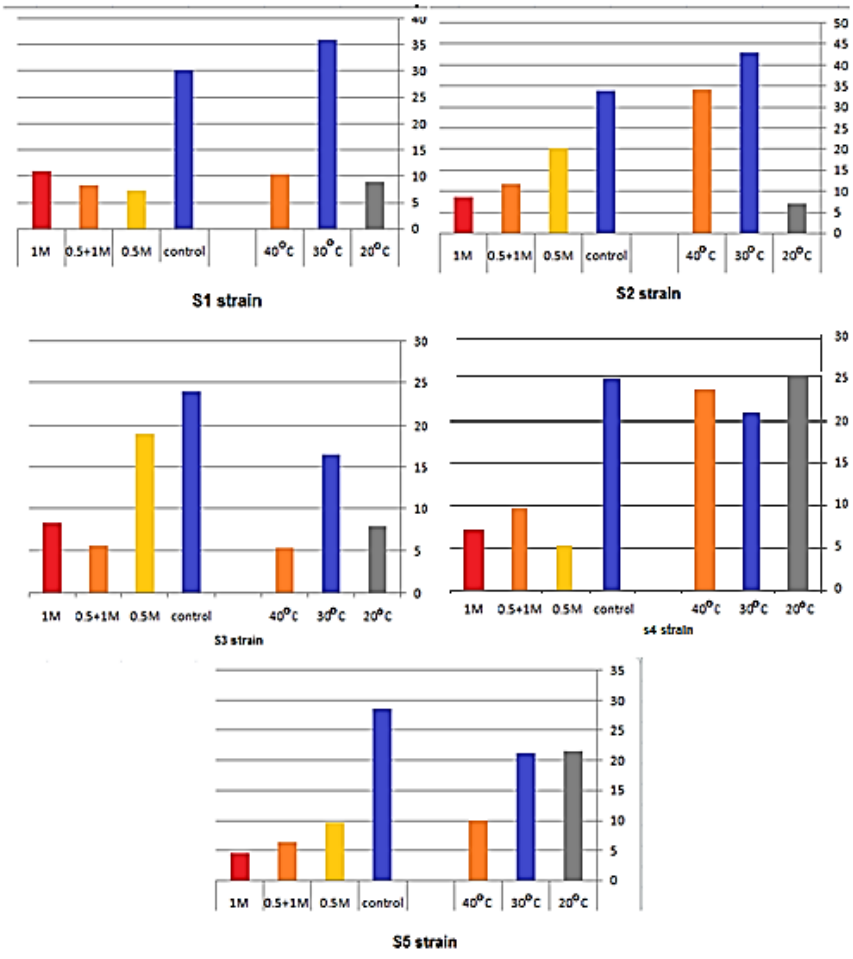

Fig. 1. Mean number of colonies that exposed to temperatures 20^{o}C and 40^{o}C and salinity at concentration (0.5,0.5+1M and 1 M NaCl)

Table 2. Number of colonies forming units of *Saccharomyces cerevisiae* after exposure to heat or salt stresses compared with the control.

Stress	Treatments	Rep 1	Rep 2	Rep 3	Mean
		S1			
Heat stress	20°C	11	9	7	9*
	30°C(cont.)	34	35	39	36
	40°C	12	12	7	10.3*
Salt stress (NaCl)	Control	32	28	31	30.3
	0.5 M	7	5	10	7.3*
	0.5 + 1 M	5	12	8	8.3*
	1 M	11	8	14	11*
		S2			
Heat stress	20°C	7	6	8	7*
	30°C(cont.)	42	42	45	43
	40°C	34	33	36	34.3*
Salt stress (NaCl)	Control	34	29	39	34
	0.5 M	22	19	20	20.3*
	0.5 + 1 M	7	11	17	11.6*
	1 M	5	8	13	8.6*
		S3			
Heat stress	20°C	7	10	7	8*
	30°C(cont.)	19	15	16	16.6
	40°C	10	3	3	5.3*
Salt stress (NaCl)	Control	20	24	28	24
	0.5 M	14	28	15	19
	0.5 + 1 M	5	8	4	5.6*
	1 M	8	11	6	8.3*
		S4			
Heat stress	20°C	23	27	26	25.3*
	30°C(cont.)	20	23	21	21.3
	40°C	22	24	24	23.3
Salt stress (NaCl)	Control	15	27	32	24.6
	0.5 M	2	6	8	5.3*
	0.5 + 1 M	7	5	17	9.6*
	1 M	10	6	6	7.3*
		S5			
Heat stress	20°C	26	21	18	21.6
	30°C(cont.)	20	21	23	21.3
	40°C	10	11	9	10*
Salt stress (NaCl)	Control	23	36	27	28.6
	0.5 M	10	7	12	9.6*
	0.5 + 1 M	2	8	9	6.3*
	1 M	3	6	5	4.6*

* = Significant differences for treatments compared with the control (Dunnett Test).

These results were confirmed by the previous findings of **Wei et al (1982)** who reported that the effect of high salt content on semisolid culture is essentially the same as the effect on liquid culture; i.e., as the salt content increased, the following occurred: (1) the growth of yeasts decreased, (2) the lag period of the yeast biomass curve lengthened, (3) the sugar intake was lowered, (4) the yield of ethanol was reduced.

Logothetis et al (2007) also, found that salt pre-treatments resulted in beneficial influences on both cell viability and fermentation performance of an industrial yeast strain**.**

CONCLUSION

Yeast strains showed different responses under heat and salt experiments when compared with the control. The detection of three strains tolerant to heat or salt stresses are a good trail that works may be completed on these strains to study molecular genetics effect at the level of protein and DNA.

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استنباط بعض سلالات الخميرة (*ساكارومايسس سيريفسيا*) المتحملة لإجهادى الملوحة والحرارة [54]

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الموجـــــز

تتعرض جميع الكائنات الحية لظروف بيئية متغيرة يجب عليها التكيف معها، حيث يتم تعريف الإجهاد على أنه تهديد للتوازن، وهو التوازن الفسيولوجي للأنظمة الحيوية للبقاء على قيد الحياة. يوجد الآن تقدم كبير أدى الى فهم الآليات المختلفة المتسببة في استجابة الخميرة للإجهاد البيئي. تخضع هذه الخميرة لأوضاع بيئية مختلفة، مثل الضغط الاسموزي، والحمضي والأكسدة. ومع ذلك، هناك أيضا مسارات استجابة محددة تتضمن الإجهاد الاسموزي، الإجهاد الحراري. تم تعريض خمس سلالات من الخميرة لضغوط مختلفة، من حيث تغيير درجة حرارة النموحيث تم تم تعريضها للنمو عند 20 درجة مئوية أو 40 درجة مئوية إلى جانب عينة المقارنة عند 30 درجة مئوية. وقد تم أيضا تم تنمية السلالات في تركزين مختلفتين من الملوحة (0.5 أو 1 مولار) لمدة يومين. كما أجريت تجربة أخرى بتركيز قدره 0.5مولر لمدة 24 ساعة ثم 1مولار لمدة 24 ساعة أخرى عند 30 درجة مئوية (درجة الحرارة الطبيعية لنمو السلالات) وذلك لتحديد أكثر السلالات تحملا للملوحة. وقد أوضحت النتائج أن السلالة S4 أكثر تحملا عند درجة حرارة 40°م حيث كان الفرق غير معنوى بينها وبين عينة المقارنة كما تحملت النمو أيضاً عند درجة حرارة 20°م بعدد مستعمرات أعلى معنوياً من عينة المقارنة. وكانت السلالة S5 أكثر تحملاً عند درجة 20°م بفرق غير معنوي عن عينة المقارنة، وبالنسبة لتجربة تحمل الملوحة وجد أن السلالة S3 تحملت تأثير الملوحة بتركيز 0.5 مولار من كلوريد الصوديوم بفرق غير معنوي مقارنة بعينة المقارنة. ساهم هذا البحث إلى تحديد 3 سلالات خميرة متحملة للحرارة أو الملوحة يمكن استخدامها فى الدراسات الوراثة الجزيئية.

الكلمات الدالة: الخميرة، *ساكارومايسيس*سيرفسيا، التكيف، الإجهاد الاسموزي، الإجهاد الحراري.

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