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ACCUMULATION OF SELENIUM BY DIFFERENT YEAST CELLS

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Abstract

Selenium (Se) may play a beneficial role in multi-factorial diseases with genetic and environmental linkages via regulation seleno-proteins activity. The supply of food supplemented with Se-enriched yeast is one of the ways to overcome deficiency of this microelement, so in this paper, the effect of concentration of sodium selenite and incubation periods on selenium bioaccumulation in yeast cells (five strains of Saccharomyces cerevisiae and two strains of Candida (pseudo and tropicalis) were studied. Yeast cells are able to bind ionic elements from the environment and incorporate them permanently into their cellular structure. The study process factors were as follows: the kind of strains (five strains of S. cerevisiae and two strains of Candida (pseudo and tropicalis), incubation period and Se concentration (0, 0.5, 1.5, 3.0, 4.5, and 6.0 mM). After incubation period at 30°C, the content of selenium in the yeast was determined by the fluorometric method using atomic absorption spectrometer. It was demonstrated that the yeast intracellular selenium concentration increased and the yeast dry biomass yield decreased along with an increased Se concentration in the culture medium depending on the periods of incubation, the accumulation of Se into the yeast *S. cerevisiae* MT3 was 9.1 mg/g of dry biomass. The highest amount of cellular selenium was 8.2 mg/g of dry biomass for Candida pseudo yeast.

Keywords: Yeast, *Saccharomyces cerevisiae*, *Candida*, selenium, precentage of survival, biomass yield.

Introduction

The trace mineral Se is a vital component of all living organisms and its deficiency is considered to be important in various types of cancer. Se deficiency can lead to many diseases, including neurological, Keshan, or Kashin – Beck disease (Kieliszek and Błazejak, 2016). This element is a component of some important selenoproteins and enzymes required for main functions in organisms as antioxidant defense, reduction of inflammation, thyroid hormone production, DNA synthesis, fertility, reproduction (Rayman, 2000). It was first recognized the physiological significance of Se by the evidence that Se is an essential component of glutathione peroxidase with important antioxidant and detoxifying functions (Zeng and Combs, 2008; Zhou *et al.,* 2009). Selenium is the primary source of food. While selenium in food and feed is varied, selenium yeast preparations enriched with organic selenium are among the most efficient and safe supplementation methods at the same time. Their application has beneficial effects on human health multidirectionally (Kieliszek and Błazejak, 2013).The most bio-available for human and animal use are complexes of organic selenium and selenium-containing amino acids (Zhou *et al.,* 2009).

The use of yeasts in human nutrition as Se supplement has been gaining a lot of attention over the past decade. Several researchers are particularly interested in the biochemical transformation and accumulation of Se in yeast cells (Kieliszek *et al.,* 2017). Under appropriate conditions, yeasts may accumulate and integrate large quantities of trace elements such as Se into organic compounds (Suhajda *et al.,* 2000), mainly Se-Met (Choi *et al.,* 2002). Na_2SeO_3 can be bio-transformed to organic form and being absorbed by the yeast (Combs and Lu, 2001). Through this process, inorganic selenite can be converted as a low bioavailable toxic component with improved nutritional properties to safer, highly bioactive species. SeMet is the main form of Se in yeast cells, but it depends on the conditions of culture and the strain of the yeast (Suhajda *et al.,* 2000). *S. cerevisiae* is only yeast strain that has been used by manufacturers for production of Se-enriched yeast (E.F.S.A, 2008). The aim of this work is to study the effect of concentration of sodium selenite and incubation periods on Se bioaccumulation in yeast cells (five strains of *S. cerevisiae* and tow strains of *Candida* (*pseudo* and *tropicalis*).

Materials and Methods

Yeast strains (*Candida pseudo*, *Candida tropicalis*, *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* ARC, *Saccharomyces cerevisiae* MF and *Saccharomyces cerevisiae* MT3) were obtained from NRC Cairo Egypt. Sodium selenite, yeast extract, peptone, dextrose and agar. The following components were included in the YEPD medium used in these studies (yeast extract, 3 g/L; peptone, 10 g/L; dextrose, 20 g/L distilled water) at pH 5.5.

Yeast strains were inoculated from agar slants in 10 ml of YEPD medium, and shacked overnight (ON) at 30 ^{o}C, then streaked on agar plates, this culture was incubated at 30 ^{o}C for 24 – 48 hours (Kaur and Bansal, 2006).

Cultures and Strains

To test the tolerance of yeast strains (7 strains) to high levels of Se, agar plates of yeast mold (YM) with different levels of inorganic Se (sodium selenite) were used. The cultures of strains were prepared using Hyo-Youn *et al.,* 2013 method.

Determination of cellular Se concentration

Se was measured by (A Varian Model Spectr AA220 atomic absorption spectrometer) using the Association of Official Analytical Chemists (AOAC) Official Method 2006.03 with some modifications (Kane and Hall, 2006).

Determination of Se using Flame Atomic Absorption Spectrometry

Flame atomic absorption spectrophotometer with four hollow cathode lamp positions was used (SpectrAA220, Varian, Australia).Depending on the measured element, the light beam through Air-Acetylene was controlled by an aperture to measure absorbance at a different slit width. The oxidant rate was 4.5 L / min and a fuel rate of 1.5 L / min (C2H2). In the present study, the light sources were a Se hollow-cathode lamp filled (37 mm Varian Coded, Cathoden, UK). The hollow cathode lamp was filled with Ne inert gas and attached with quartz window. The most sensitive absorption line of Seat 196.0 nm was used. In order to obtain maximum sensitivity, the optimum operating conditions in terms of absorbance mode, measuring time, burner height, gas flows or amplifier gain were optimized. Fresh standard solutions of Se were prepared daily and used (Merck, Germany). The background correction was carried out using a continuum source of deuterium arc lamp in ultraviolet region. Using area absorbance mode, the absorbance values of the standards and samples were measured five times and the average value was used.

Determination of yeasts dry cell weight

The weight of dry cell had been determined by the method of Hyo-Youn *et al.,* 2013.

Screening of the cellular Se content in selected yeast

This experiment was designed for Screening of the cellular Se content in selected yeast strain cultures after 72h treatment with different concentrations of sodium selenite. Different quantities of sterilized sodium selenite have been applied in this process to Erlenmeyer flasks containing 50 ml basal medium at zero time (after inoculation) to give 0.5, 1.5, 3.0, 4.5 and 6.0 mM sodium selenite in water, in addition to controlling one without medium sodium selenite. After that these flasks were inoculated with prepared starter of selected yeast strains and then incubated at 30°C and 180 rpm for 72 h. At the end of fermentation time, yeast cells were centrifuged, washed and dried at 70°C to a constant weight, after that content of organic selenium were measured. Triplicate measurements were conducted and the results were averaged (Mona *et al.,* 2015).

Effect of various concentrations of sodium selenite on survival percentage of different yeast strains

This experiment was designed to study the effect of different concentrations of sodium selenite (0.5, 1.5, 3.0, 4.5 and 6.0 mM) in addition to control one without sodium selenite on survival % of different yeast strains at different cultivation time 48 and 72 h using previous method. After cultivated at 30°C for 48 and 72 h, viable cells were enumerated before (initial count) and after addition different concentrations of sodium selenite. Colonies per plate were counted at the interval of 30–300 colony-forming units. Both tests have been carried out in duplicate. The survival rate has been determined as follows:

$$\text{Viability} = N / N_0 \times 100$$

where N and N_0 represent the viable counts (cfu/g) after incubation with different concentrations of sodium selenite and the initial count (cfu/g) before treated with sodium selenite, respectively.

Effect of various concentrations of sodium selenite on *S. cerevisiae* MT3 and *Candida pseudo*

This experiment was designed to study the effect of different concentrations of sodium selenite (zero, 0.3, 0.6, 0.9, 1.2 and 1.5 mM)on cellular Se content, biomass yield and survival percentage at different cultivation time 48 and 72 h to *S. cerevisiae MT3* and *Candida pseudo* by using the previous method.

Results

The first table showed that seven yeast strains (two *Candida* and five *S. cerevisiae*) have been tested for their ability to accumulate Se in the cell wall by grown them under different concentrations of selenium (sodium selenite) at concentrations of 0-6 mM for three days.

Table (1) showed that with increasing concentration of sodium selenite from 0-1.5 mM, the accumulation of cellular Se increased in all tested yeast cells. The *S. cerevisiae* MT3 strain was the highest yielding about 224.47 µg / g dry weight at 1.5 mM sodium selenite followed by *C. pseudo* which possessed yield 147.26 µg / g dry weight at 6.0mM sodium selenite concentration.

Table 1 : Screening of the cellular selenium content in selected yeast strain cultures after 72h treatment with different concentrations of sodium selenite.

Strains	Concentration of sodium selenite in medium (mM)					
	zero	0.5	1.5	3.0	4.5	6.0
	Concentration of cellular Selenium (µg/g dry weight)					
Candida pseudo	1.47	53.88	95.67	94.43	93.30	147.26
*S. cerevisiae*2	0.54	4.26	8.95	09.95	09.68	13.11
S. cerevisiae	0.18	4.39	9.77	23.10	09.02	18.16
S. cerevisiae ARC	0.18	2.82	4.92	08.46	07.55	09.43
C. tropicalis	0.13	4.10	13.04	50.89	41.18	52.99
*S. cerevisiae*MF	0.13	2.76	5.115	04.63	08.09	12.80
S. cerevisiae MT3	0.126	23.61	224.47	43.38	43.29	36.67

The effect of various concentrations of sodium selenite on the percentage of survival % of the yeast cells and its development at different periods ranging from 48-72 hours was shown in Table (2). It was noted that the percentage of survival rate decreases with increasing the concentration of sodium selenite, but the degree of decrease varies depending on the type of yeast. The highest of survival rate was about 92.60 % for *S. cerevisiae* MT3 after 48 hours under a concentration of 0.5 mM of sodium selenite.

Table 2: Effect of various concentrations of sodium selenite on survival % of different yeast strains at different cultivation time 48 and 72 h.

Strain	Concentration of sodium selenite in medium (mM)											
	zero		0.5		1.5		3.0		4.5		6.0	
	Survival%											
	48h	72h	48h	72h	48h	72h	48h	72h	48h	72h	48h	72h
C. pseudo	100	100	63.3	71.80	52.3	63.9	40.4	43.80	13.80	23.40	0.46	11.60
S. cerevisiae 2	100	100	70.3	74.73	55.6	53.4	44.4	43.04	15.30	42.51	nd	91.00
S. cerevisiae	100	100	12.9	51.92	10.8	33.8	12.2	21.67	10.80	18.51	0.66	10.39
S. cerevisiae ARC	100	100	79.0	55.60	12.2	62.6	02.2	49.60	nd	17.33	nd	03.80
C. tropicalis	100	100	84.2	89.33	84.2	72.0	81.9	74.66	79.40	73.33	nd	54.66
S. cerevisiae MF	100	100	92.0	89.89	49.0	37.5	07.6	08.99	00.26	04.27	0.15	00.15
*S. cerevisiae*MT3	100	100	92.6	77.40	16.5	71.9	09.7	05.40	00.21	08.80	0.10	11.49

nd= not detected

Table (3) represented the effect of concentration of sodium selenite and cultivation period on the biomass and percentage of survival of *S. cerevisiae* MT3.The results showed that there was no relationship between biomarker growth or biomass and increase the concentration of sodium selenite in the cell wall. The highest accumulation of cellular Se was 9.19 mg / g while the biomass was 1.93 g / L and the survival % was 21.62% at cultivation period 48 h and the concentration of sodium selenite was 1.5 mM , followed by 7.66 mg/g cellular selenium when the biomass was 2.21 g/L and 39.1% survival after 72-hour development at a concentration of 0.9 mM sodium selenite.

Table 3: Effect of various concentrations of sodium selenite on cellular Se content, biomass yield and survival % at different cultivation time 48 and 72 h to *S. cerevisiae* MT3

Sodium selenite in medium (mM)	*Time*					
	48h			72h		
	Conc. of cellular Se (mg/g)	Biomass yield (g/L)	Survival %	Conc. of cellular Se (mg/g)	Biomass yield (g/L)	*Survival%*
Zero	0.15	5.35	100.00	2.79	7.11	100.00
0.3	4.30	2.13	94.59	5.55	1.98	63.04
0.6	3.89	2.11	78.30	4.21	1.85	36.96
0.9	3.40	2.05	59.46	7.66	2.21	39.10
1.2	8.01	2.09	19.46	6.60	1.84	14.56
1.5	9.19	1.93	21.62	7.68	2.05	13.04

The effect of various concentrations of sodium selenite on cellular Se content, biomass yield and survival % at different cultivation time 48 and 72 h to *C*andida pseudo was illustrated in Table (4).The results showed that the highest accumulation of cellular Se was 7.32 mg / g with 3.92 g / l biomass and 7.60 % of survival at 1.5 mM sodium selenite after 48 h but 4.63 mg / g of cellular Se was accumulated with 4.64 g / L biomass and 47.90% survival at a concentration of 0.6 mM sodium selenite.

Table 4: Effect of different concentrations of sodium selenite on cellular Se content, biomass yield and survival % at different cultivation time 48 and 72 h to *C. pseudo*

Sodium selenite in medium (mM)	*Time*					
	48h			72h		
	Conc. of cellular Se (mg/g)	Biomass yield (g/L)	Survival %	Conc. of cellular Se (mg/g)	Biomass yield (g/L)	*Survival%*
Zero	0.19	6.05	100.0	0.29	4.79	100.0
0.3	2.18	5.41	70.0	3.07	4.87	nd
0.6	4.07	4.21	40.7	4.63	4.64	47.9
0.9	5.41	4.04	29.6	8.20	4.02	39.4
1.2	5.03	3.76	20.3	7.87	3.78	19.6
1.5	7.32	3.92	07.6	7.4	3.91	09.7

nd = not detected

Discussion

S. cerevisiae do not contain seleno-proteins and therefore Se is not essential for these organisms (Lu and Holmgren, 2009). When Se feeding is not carefully controlled, Se can be toxic to yeast cells since it generates oxidative stress and even provokes DNA damage (Lewinska and Bartosz 2008; Letavayová *et al.,* 2006). Both the type of sugar and the concentration of Se in the growth medium significantly influence ($p < 0.001$) Se content in baker's yeast cells, and Se concentration in the yeast growth medium affects the yeast dry biomass yield. *S. cerevisiae* yeast biomass after fermentation constituted between 7.02 and 9.82 g. The highest biomass production was obtained in the media

containing glucose. (Beata *et al.,* 2018) yeast cells did not absorb all ions present in the culture medium, even in the case of their low concentration. Accumulation of metal ions depends probably on the intracellular transportation systems and on their chelating strength by the medium's compounds and cellular substances (Tuszyński and Pasternakiewicz, 2000). Intracellular Se phase accumulation occurred through active transport within yeast cells. A unique transportation mechanism was needed to overcome the cell membrane impermeability of Se ions. To date, only a few studies have been published that explain this process in yeast cells. In addition, there are no studies at the molecular level on the detection of Se carriers (Rosen and Liu 2009). Study of available research shows that many studies related to the study of intracellular accumulation of Se primarily apply to yeast. Metabolism and accumulation of Se in yeast cells are a very complex process (Gharieb and Gadd, 2004).

Conclusion

The aim of the study was to produce yeast enrichment with Se. For the ability to use Se enriched biomass yeast to produce protein and mineral preparations which can be used as supplements to resolve diet deficiencies. The use of different kind of Se concentrations in a medium culture resulted in the accumulation of Se into the yeast cells in the range of mg g^{-1}. In this study increasing the amount of Se addition decrease the inhibition of yeast cell growth, although the total Se concentration in yeast cells increases with increased Se added to the cultivation media.

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