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Selenium enrichment and anti-oxidant status in baker's yeast, *Saccharomyces cerevisiae* at different sodium selenite concentrations

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Abstract

The use of selenized yeast as enriched selenium supplements in human nutrition has become a topic of increasing interest over the last decade. The present study was designed with the aim to achieve a balance between selenium (Se) incorporation and optimal growth of yeast cells along with effect of Se enrichment on antioxidant defense status of yeast cells. Since oxidative stress has been known to play a role in the life span of all types of cells, so in the present studies anti-oxidant defense status was evaluated in the Se- enriched baker's yeast cell culture model. Upon Se supplementation as sodium selenite at various concentrations in the growth medium, a continuous increase in glutathione peroxidase (GSH-Px) activity and Se content was observed. In case of reduced glutathione (GSH) decreasing trend were observed with increasing Se concentrations. An increasing trend in total glutathione as well as glutathione-s-transferase activity was observed at increasing Se concentrations. Thus, Se supplementation significantly enhanced GSH-Px levels along with alterations in other anti-oxidant enzymes, suggesting the role of Se in the enzyme defense system of yeast against oxidative damage. Further, as Se exerts growth inhibitory effect on cells, the growth inhibition study was carried out and decrease in biomass was observed with increasing concentrations of Se. Due to nutritional benefits, Se-enriched yeast may be considered a safe source of Se supplementation.

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Key words: *Sodium selenite. Selenium uptake. Biomass. Anti-oxidant system.* Saccharomyces cerevisiae.

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ENRIQUECIMIENTO CON SELENIO Y ESTADO ANTI-OXIDANTE DE LA LEVADURA DE HARINAS *SACCHAROMYCES CEREVISIAE* CON DIFERENTES CONCENTRACIONES DE SELENITO SÓDICO

Resumen

El uso de levaduras "selenizadas" como suplementos enriquecidos con selenio en nutrición humana se ha convertido en un tema de interés creciente en la última década. Este estudio se diseño con el objetivo de conseguir un equilibrio entre la incorporación de selenio (Se) y el crecimiento óptimo de las células levaduriformes, junto con el efecto del enriquecimiento de Se sobre el estado de defensa anti-oxidante de las levaduras. Puesto que se sabe que el estrés oxidativo desempeña una función en la longevidad de todo tipo de células, en este estudio se determinó pues el estado de defensa anti-oxidante en un modelo de levadura de la harina enriquecida con selenio. Tras la complementación con Se, en forma de selenito sódico, en concentraciones variables en el medio de cultivo, se observó un aumento sostenido de la actividad glutatión peroxidasa (GSH-Px) y del contenido en Se. Con respecto al glutatión reducido (GSH), se observó una tendencia a la baja a medida que aumentaban las concentraciones de Se. Se observó una tendencia al alza del glutatión total y de la actividad glutatión-s-transferasa a medida que aumentaba la concentración de Se. Por lo tanto, la complementación con Se favoreció de forma significativa las concentraciones de GSH-Px junto con cambios en otras enzimas anti-oxidantes, lo que sugiere un papel del Se en el sistema enzimático de defensa de la levadura frente al daño oxidativo. Además, puesto que el Se ejerce un efecto inhibidor del crecimiento celular, se realizó un estudio de inhibición del crecimiento y se observó un descenso de la biomasa con concentraciones crecientes de Se. Dados los beneficios nutritivos, se podría considerar la levadura enriquecida con Se como una fuente segura de complementación de Se.

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Palabras clave: *Selenito sódico. Ingestión de selenio. Biomasa. Sistema anti-oxidante.* Saccharomyces cerevisiae.

Introduction

The trace element, selenium (Se), has been well recognized as being essential in animals and humans[1]. Se deficiency has been associated with cases of congestive cardiomyopathy, skeleton myopathy, anemia, enhanced cancer risk, elevated levels of cardiovascular diseases, immune system alterations and abnormalities in thyroid hormone metabolism[2]. Its main physiological role is connected with its presence in various selenoenzymes basically involved in redox type reactions[3]. The extent of the biosynthesis of selenoproteins with molecular masses of 73 and 83 kDa in mitochondria and cytosol of *Saccharomyces uvarum* has been shown to depend on the sodium selenite concentration in the medium[4]. A dietary deficiency in Se can increase the sensitivity of a living system to oxidative stress and there are reports showing oxidative stress influencing the life span of various yeast strains especially those related with deficiencies in various anti-oxidant mechanisms[5,6].

Brewer's and baker's yeast *(Saccharomyces cerevisiae)* has been used in classical food fermentation applications (beer, bread, yeast extract/vitamins, wine, saké, distilled spirits). It is also used in the production of fuel alcohol, glycerol, invertase and animal feeding, thus suggesting the multiple potential uses of baker's yeasts[7]. Selenized yeast has been the most widely investigated natural product containing selenium. The interest in these studies was triggered off by the study of Clark y cols., who indicated a possible role of selenized yeast in cancer prevention[8,9]. Epidemiological evidences are now emerging for beneficial effects of Se-enriched yeast supplementation. Unfortunately, modern farming techniques and refined food diets leads to reduced Se status[10]. Therefore, close similarity of selenized yeast to the natural forms found in feed crops, plus the careful control of Se content that can be exercised in yeast production, makes Se-yeast, a most interesting, useful and environmentally-safe supplementary material for use with livestock. In the present study, to better understand the biological consequences of alterations in Se status, sodium selenite was used at various concentrations to elucidate the effect on levels of anti-oxidant system along with the growth inhibition study. Technically, yeast are simple to handle, inexpensive to grow, complete a cell cycle within 90 minutes[11] and therefore, can yield quick results, thus making it a successful model organism for the present work.

Materials and methods

Yeast strain, *Saccharomyces cerevisiae* (MTCC Code-1766) was obtained from Institute Of Microbial Technology, Chandigarh (India). Sodium selenite, yeast extract, peptone, dextrose and agar were obtained from Sigma Chemical Co (St. Louis, MO, USA). All other chemicals used were of Analytical Grade from other Indian Manufacturer.

YEPD medium used in the present studies contained the following components: Yeast extract, 3 g; peptone, 10 g; dextrose, 20 g in one liter of distilled water; final pH 5.5. The yeast strain was maintained on the YEPD agar slants (1.5% agar). Yeast, *Saccharomyces cerevisiae,* was inoculated from agar slants in 10 ml of YEPD medium and incubated overnight (ON) at 30° C in shaker. This culture was streaked on agar plates and incubated at 30° C for 24-48 hours. Further, one colony was inoculated in fresh YEPD medium and incubated ON at 30° C on shaker. The culture so obtained contained approximately 1.6 x 10^5 cells/ml as determined by haemocytometer counts.

Selenium supplementation to culture

Selenium in inorganic form as sodium selenite (Na_2SeO_3) was used. From 25 mM sodium selenite stock solution 19, 39 and 57 μM Se concentrations were made in different 20 ml aliquots of yeast culture media. Media without addition of Se was used as control. These medias with different Se concentrations were inoculated with 100 µl of ON culture and incubated at 30°C for ON growth on shaker. After incubation, growth pattern of yeast cells was studied by taking O.D at 600 nm. Se was estimated in cell pellet from 20 ml ON culture[12].

Preparation of cell extract

Cell pellet of 20 ml ON culture obtained after centrifugation at 2,000 rpm for 15 min was washed two times with 3 ml of Tris-HCl buffer (50 mM, pH 7). Further, cells were resuspended in 3 ml of Tris-HCl buffer and disrupted along with glass beads (0.2 mm in diameter) using vortex mixture. The cell extract obtained was centrifuged at 10,000 g for 15 min at 4° C and supernatant was used for evaluation of anti-oxidant system comprising of glutathione peroxidase (GSH-Px) enzyme activity[13], total and reduced glutathione[14], glutathione-S-transferase[15] and protein concentration[16].

Anti-oxidant Studies

Briefly, GSH-Px enzyme activity was measured by recording the amount of NADPH oxidized using 340 nm absorbance and H_2O_2 as substrate. For total glutathione estimation, reduced sample with dithiothreitol in presence of arsenite was reacted with 5,5'-thiobis-2-nitrobenzoic acid (DTNB) and then quantitative at 412 nm. Similarly, for reduced glutathione, samples were directly treated with DTNB and quantitated. For glutathione-S-transferase estimation, conjugation of 1-chloro-2, 4-dinitrobenzene (CDNB) and glutathione (GSH) by the enzyme was quantitated at 340 nm. The protein content was estimated by the standard method of Lowry y cols. (1951).

Statistical analysis

Results are expressed as Mean ± SEM of four observations. Data were analyzed to determine statistical

significance by student's t-test using Sigma Stat statistical software version 2.0.

Results

Yeast, *Saccharomyces cerevisiae,* was inoculated in YEPD medium with inorganic form of Se as sodium selenite at various Se concentrations (viz. 19, 39 and 57 μM Se) and incubated at 30° C for 24 h. Cell growth, selenium content and glutathione peroxidase levels were analyzed and results are shown in table I. A significant decrease ($p < 0.001$) in growth of yeast cell in terms of biomass (A_{600}) was observed. This decrease in cell growth was dose-dependent with increasing Se concentrations. The Se content in yeast cells was found significantly increased ($p < 0.001$) with increasing concentration of Se in the medium viz. 453% at 19 μM, 593% at 39 μM and 910% at 57 μM Se. GSH-Px activity also was found to increase significantly with increasing Se concentration in the medium viz. 174% at 19 μM, 420% at 39 μM and 560% at 57 μM Se.

Table II shows the levels of glutathione, redox ratio and glutathione-s-transferase at different Se concentrations. Se supplementation resulted in an increasing trend of total glutathione, viz. 12% at 19 μM, 20% at 39 μM and 31% at 57 μM Se. Whereas, in case of reduced glutathione (GSH) decreasing trends were observed with increasing Se concentrations. Reduced glutathione decreased to 21% at 19 μM, 58% at 39 μM and 68% at 57 μM Se. Also, the "Redox Ratio", GSH/GSSG, decreased with increasing Se concentrations. Increasing trend in glutathione-s-transferase activity was observed in yeast extract of *Saccharomyces cerevisiae,* at increasing Se concentrations. At maximum Se concentration, significant increase of 374% at 57 μM Se was observed with sodium selenite supplementation.

Discussion

Se is of fundamental importance to health and has been identified as an important antioxidant in the diet. As a result of agricultural practices, low Se soil contents and dietary preferences, adult populations in most western industrialized nations presently reach Se intakes of only one-third of this amount. Animal experiments have shown that for maximum protective effect, Se supplementation has to be maintained over the entire life span. Use of Se as nutritional supplement

Table I

Influence of three different concentrations of sodium selenite on yeast cell growth, Se content and glutathione peroxidase activity after overnight incubation in YEPD media (values are mean ± SEM of four observations)

Sodium Selenite concentrations (μM)	*Cell growth (A 600)*	*Selenium content (μg Se/g tissue)*	*GSH-Px activity (μmol NADPH (oxidized/min/mg protein)*
Control	0.56 ± 0.012	0.86 ± 0.12	16.15 ± 1.13
19.9	0.43 ± 0.017[a]	4.76 ± 0.03[a]	44.24 ± 1.37[a]
39.0	0.37 ± 0.017[a]	5.96 ± 0.01[a]	84.00 ± 1.18[a]
57.0	0.29 ± 0.019 [a]	8.69 ± 0.005[a]	106.63 ± 0.18[a]

[a]Mean values within rows are statistically significant ($p < 0.001$).

Table II

Influence of different concentrations of selenium supplemented as sodium selenite on total, reduced and oxidized glutathione, GSH/GSSG ratio and glutathione-s-transferase (GST) activity in yeast cell grown in YEPD media (Values are mean ± SEM of four observations)

Se Type	*total glutathione (μg/mg protein)*	*GSH (μg/mg protein)*	*GSSG (μg/mg protein)*	*GSH/GSSG*	*GST Activity #*
Sodium Selenite (μM)					
Control	548.0 ± 31.0	510 ± 24.7	41.00 ± 12	12.43 ± 6.8	10.08 ± 1.17
19.0	612.0 ± 25.12[b]	402 ± 60.0	210.25 ± 17.0[c]	1.91 ± 0.33[c]	20.78 ± 0.68[b]
39.0	659.0 ± 55.9[c]	215 ± 45.0[a]	444.00 ± 84.0[c]	0.48 ± 0.22[c]	31.15 ± 0.31[a]
57.0	715.0 ± 57.0[c]	164 ± 29.0[a]	550.50 ± 83.6[c]	0.29 ± 0.07[c]	47.73 ± 2.16[a]

[a]Mean values within rows are statistically significant ($p < 0.001$).
[b]Mean values within rows are statistically significant ($p < 0.01$).
[c]Mean values within rows are statistically significant ($p < 0.05$).
[#]μmoles CDNB-GSH conjugate formed/min/mg protein.

has been popularized due to its potential role in low concentrations as an antioxidant and in higher concentrations as an anti-carcinogenic agent[17]. Seeing the anti-carcinogenic activity of Se, recent attempts have been initiated to explore safest way to improve Se status in human beings especially in the form of selenized yeast. In this context, the determination of both yeast optimal growth and operating conditions for enhanced biomass production are of great concerns in food and pharmaceutical industries. In the present study, Se content, optimum growth curve and the antioxidant defense status of Se enriched yeast by sodium selenite were thus evaluated. Selenium supplementation resulted in the dose dependent increase in GSH-Px activity and Se content suggesting the role of Se in the enzyme defense system of yeast against oxidative damage. The anti-oxidative role of Se as an integral part of GSH-Px is well established. Reactive oxygen species, organic hydroperoxides and hydrogen peroxidase are known to cause genetic damage and possibly induce cancer. Both forms of enzyme, specifically active toward H_2O_2 alone and that decomposing organic peroxides, have been found to be present in the wild type strain of *Saccharomyces cerevisiae*[18]. Selenium acts through its enzymes, cytosolic glutathione peroxidase or membrane bound phospholipid hydroperoxide glutathione peroxides and thioredoxin reductase to control levels of cellular hydroperoxides and the redox tone of cells that can damage proteins, cell and organelle membrane and DNA[19]. In many tissues of different species, the availability of GSH-Px is reflective of Se availability[20] and GSH-Px has been widely used as indicator of Se status. Moreover, the partial characterization of the anti-oxidative defenses in bakers yeast, *Saccharomyces cerevisiae,* has confirmed the presence of superoxide dismutase, catalase, glutathione and peroxidase.

Apart from changes in the GSH-Px levels, the decrease in redox ratio (GSH/GSSG) was observed at various concentrations of selenite supplementation. It is well documented that glutathione can react with oxidative agents and is involved in the oxidative stress response through GSH-Px. In the yeast, glutathione (L-gamma-Glutamyl-L-Cysteinylglycine) has been reported to be the major non-protein thiol compound[21]. Recent advances have shown that glutathione (GSH) seems to be involved in response to different nutritional and oxidative stresses and when the yeast, *Saccharomyces cerevisiae,* is starved for sulfur or nitrogen nutrients, GSH may be mobilized to ensure cellular maintenance[22]. In yeast, *S. cerevisiae,* GSH and catalase also have been shown to provide overlapping defense system.

Glutathione-S-Transferases are mainly involved in the free radical scavenging and peroxide reduction through the formation of GSH conjugates[23]. Thus, in present study increase in this enzyme by Se may be involved in the detoxification of electrophilic xenobiotics. Aniya and Anders[24] have suggested that regulation of this enzyme may be dependent on mixed disulphide formation, which in turn is controlled by GSH and GSSG levels. In the study by Christensen et al[25] both the Se deficiency as well as excess resulted in an elevated glutathione-s-transferase levels. The increased level in glutathione-S-transferase in our result may be reflecting the increase in some different subclass of the enzyme.

It was observed that with increasing concentrations of Se as sodium selenite, there was a significant dose-dependent decrease in cell growth (A_{600}). Le Bouef et al (1985) also showed the dose-dependent effect of Se on cell proliferation. The decrease in cell growth can be attributed to selenium's role as a toxic and growth-inhibiting element by formation of selenium binding proteins (SBP) and/or by modulating properties of growth regulatory protein. Many Se compounds have dramatic affects upon the viability of the cells, on the cell cycle, on protein synthesis and on DNA integrity when studied in cell culture[26]. Selenite changes the glutathione, GSH: GSSG ratio via GSH oxidation, inhibiting the G1, G2 and S-phases of cell division and protein synthesis[27]. In addition to affecting enzymes, selenite can cause DNA damage and induce apoptosis. Anti-oxidative defenses working against oxidative stress can however improve the life span of yeast cells. Studies by Wawryn et al (1999) have shown that deficiencies in superoxide dismutases result in an almost threefold shortening of the life span of individual yeast cells[28]. Thus, enhanced anti-oxidative defense status seems to be beneficial for the growth and survival of yeast cells.

During the growth of *S. cerevisiae* yeast, selenite, which is a potentially toxic and poorly bioavailable species, is converted and enriched into a safer and highly bioactive species with improved nutritional properties. Thus, in the present study, enriching yeast with inorganic form as sodium selenite resulted in enhanced Se uptake. Also, the balance between Se incorporation and optimum growth of yeast cells was achieved along with the yeasts enhanced anti-oxidative defense status. Se enriched yeast should be thus viewed as a safe and effective form of Se supplementation.

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