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EFFECT OF SELENIUM ON THE GROWTH OF THE YEASTS SACCHAROMYCES CEREVISIAE AND CANDIDA TROPICALIS

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Key words: sodium selenite, *Candida tropicalis*, *Saccharomyces cerevisiae*.

The effect of sodium selenite on cell number increase was studied in the yeasts *Saccharomyces cerevisiae* and *Candida tropicalis*. It was found that *C. tropicalis* was more susceptible to the toxicity of selenium than *S. cerevisiae*. The presence of selenium in the medium did not reduce the yeast' demand for sulfate ions, and the magnitude of the toxic selenium dose depended on the sulfur in the medium and on the kind of yeast.

Microorganisms exhibit various tolerances to selenium compunds. In most cases these compounds have a toxic effect on microorganism cells. Numerous studies have demonstrated the unfavourable effect of selenium combinations on the growth of the bacteria *Escherichia coli*, *E. freundii*, *Proteus vulgaris* and *Salmonella thompson* [1, 10, 14, 15]. Fels and Cheldelin [5] were the first to observe the toxicity of selenium compounds towards yeast cells.

The severity of this toxic effect depends not only on the species and genus of the studied microorganism and on the concentration of selenium introduced into the substrate, but also on the chemical character of selenium and on the presence of other components on the medium, chiefly sulfur compounds [11].

Environmental factors also have a considerable bearing on the directions of metabolic changes of selenium [4, 6]. Some microorganisms are able to adapt to originally toxic concentrations thanks to the presence of selenium reductase which reduces selenium compounds to an insoluble form unaccessible to these organisms [13].

The mechanism of selenium toxicity has not get been researched well. Barron et al. [2] have found that the presence of selenium compounds in the substrate inhibited the activity of succinic acid dehydrogenase in yeasts, but that an addition of glutathione, methionine, sulfate and cysteine reactivated this enzyme. In view of this it may be assumed that selenium compounds interfere with sulfur analogs forming complexes with active enzyme centers thereby inactivating them. On the other hand, studies with isolated enzymes indicate that selenium analogs are easily metabolized and that they are present in proteins.

The aim of the present work was to study the effect of selenium on the growth of the yeasts *Saccharomyces cerevisiae* and *Candida tropicalis*.

METHODS

Saccharomyces cerevisiae and *Candida tropicalis* strains were cultured on a medium given by Lodder [7] enriched with thiamine hydrochloride (2×10^{-4} mg/cm^3), biotin (4×10^{-6} mg/cm^3) and calcium pantothenate (1.09×10^{-3} mg/cm^3). The acidity of the medium was adjusted to pH 4.8 by adding a buffer composed of glycin, citric acid, KH_2PO_4 and NaOH. The cultures were maintained on a rotary shaker at 28°C. After 24 h of culture the yeasts were centrifuged, washed with sterile water and transferred into flasks containing a medium devoid of sulfur compounds. Passaging was repeated three times to ensure that the yeasts exhausted their internal supply of sulfur. The medium deprived of sulfur combinations contained equivalet amounts of CH_3COONH_4 instead of $(NH_4)_2SO_4$ as the source of nitrogen, and of $MgCl_2$ instead of $MgSO_4$ as a magnesium source. Moreover, in order to obtain a greater quantity of biomass the cultures were maintained for 15 h in 10-dm^3 propagators.

Various combinations of media were applied in order to determine the effect of selenium on the growth of the studied yeast strains, namely:

— control — containing $(NH_4)_2SO_4$ and $MgSO_4$ (1.28 mg S in cm^3 medium);

— containing sulfur and selenium compounds in the ratio 605 : 1;

— containing sulfur and selenium compounds in the ratio 32.5 : 1;

— with no source of sulfur but with various concentrations of sodium selenite.

After 72 h of culture the increase in yeast cell number was assayed in a Bürker chamber and by inoculation on Petri dishes.

The following was assayed in the obtained biomass: dry mass, selenium content by the colorimetric method with 3,3'-diaminobenzidine [12], sulfur content by the method of Fischer adapted to colorimetric assay by Mecklenburg and Rosenkränzer [8].

RESULTS AND DISCUSSION

Examining the growth of *Saccharomyces cerevisiae* and *Candida tropicalis* cells on the medium without a source of sulfur but containing sodium selenite in various concentrations corresponding to contents of 1, 2, 4, 6 and 10 μg Se in cm^3 medium, it was found that each of the applied selenium additions inhibited the process of yeast cell multiplication (Figs 1 and 2).

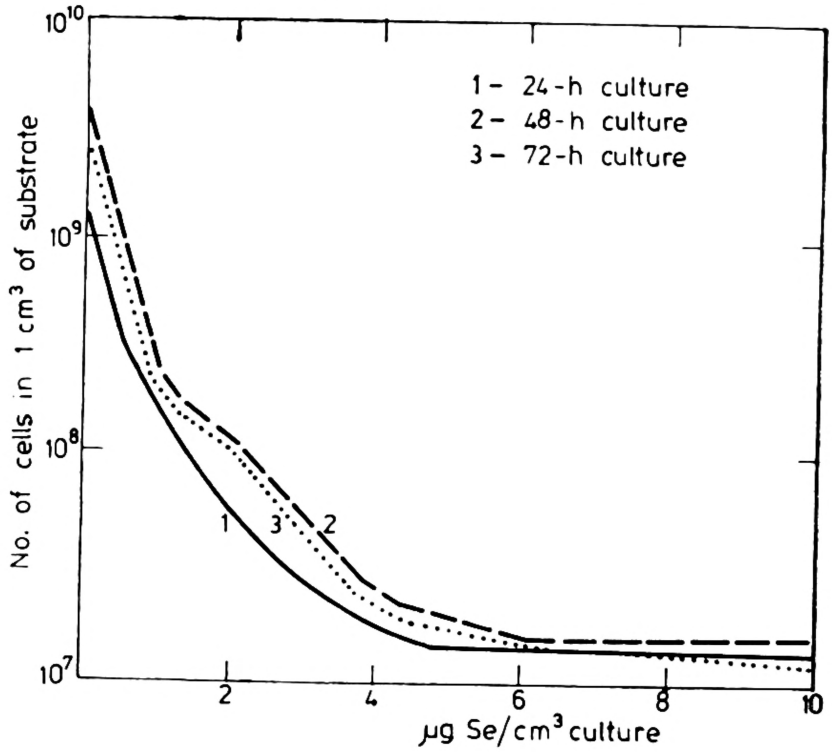

Fig. 1. Effect of selenium on the growth of *Saccharomyces cerevisiae* cells

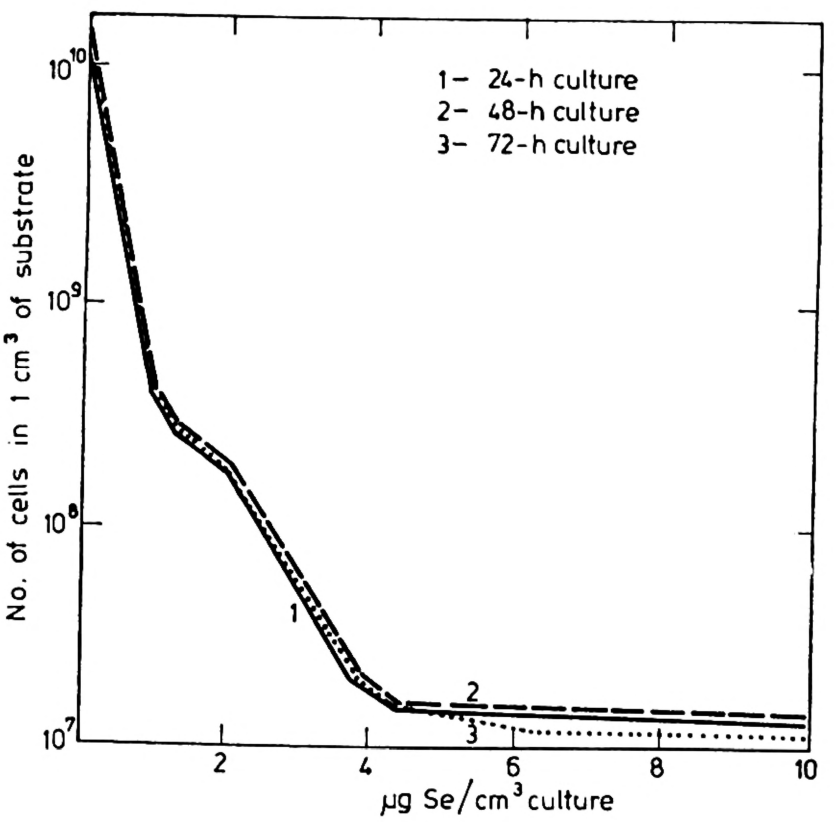

Fig. 2. Effect of selenium on the growth of *Candida tropicalis* cells

In subsequent studies of yeast growth the selenium concentration of 2 μg/cm medium was applied since at this concentration there was observed an increase in cell number, albeit very much reduced in comparison to the control culture containing no selenium. The addition of 2 μg Se/ /cm³ substrate made it possible to observe the inhibitory effect of selenium on cell number increase and to obtain a biomass yield sufficient for chemical assaying.

No clear morphological differences were found between yeast cells cultured with and without selenium. Yeast cells cultured on the substrate containing 2 μg Se/cm³ retained their ability to gemmate. *S. cerevisiae* cells cultured on the medium without sulfur and selenium and on the substrate containing selenium instead of sulfur displayed a cytoplasm structure more granular than that of cells from the control culture. Both *S. cerevisiae* and *C. tropicalis* cells cultured on the medium without sulfur and selenium were marked by a less distinct outline of cell wall than cells cultured on the medium with the two elements.

The effect of various concentrations of sulfur and selenium on yeast cell number growth is presented in Fig. 3. A selenium addition to the

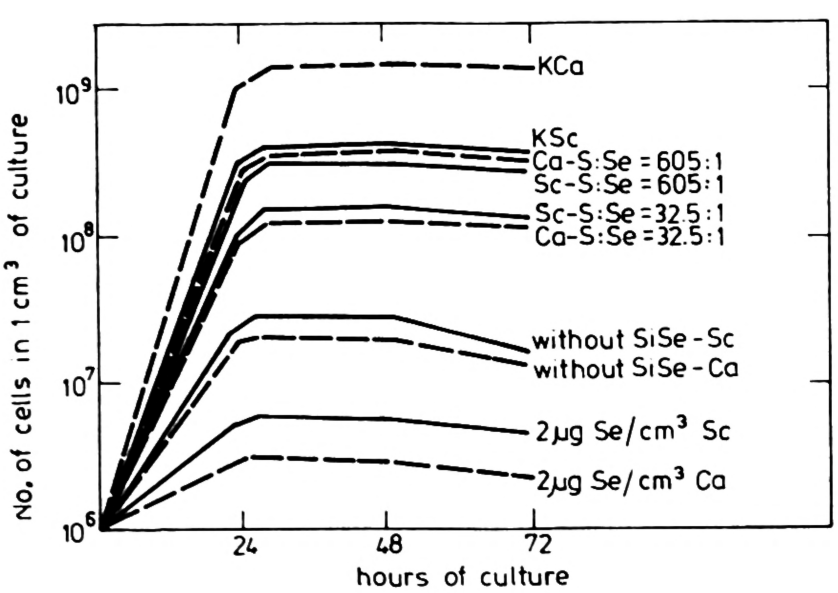

Fig. 3. Increase of yeast cell number depending on sulfur and selenium contents in the substrate; Sc — *Saccharomyces cerevisiae*, Ca — *Candida tropicalis*, K — control

medium deprived of sulfur was more inhibitory towards cell growth than the substrate without sulfur and selenium. In both cases there was a marked drop in cell number in comparison to control combinations. In media with various concentrations of sulfur and constant selenium concentration (2 μg Se/cm³) the increase of yeast cell number dropped with the reduction of sulfur concentration. At the 605 : 1 ratio of sulfur to selenium in the medium there were found 80% live cells of *S. cerevisiae* and 41% of *C. tropicalis* as compared to control cultures; at the 32.5 : 1 ratio of sulfur to selenium the respective figures were 23% and 7%. The presented data

indicate that the selenium of inorganic compounds did not reduce the yeasts' demand for sulfur.

The degree of selenium utilization was determined by studying its content in the yeasts and in the fluid from yeast centrifugation after 72 h of culture. The data in Table 1 show that increasing amounts of sodium selenite were consumed by the yeasts when the amount of sulfur in the substrate was reduced.

Table 1. Selenium content in the yeasts *Saccharomyces cerevisiae* and *Candida tropicalis* after 72 h of shaker culture

Yeast	Combination	Dry mass yield from 100 cm^3 of culture	Se content in 1 g dry mass	Se content in 100 cm^3 of fluid after yeast centrifugatio (mg)	Se utilized by yeast %
S. cerevisiae	Control 1.28 mg S/cm^3 medium	0.96	—	—	—
	S:Se 605:1	0.92	0.032	0.173	15.5
	2 μg Se/cm^3	0.29	0.502	0.053	72.8
C. tropicalis	Control 1.28 mg S/cm^3 medium	1.28	—	—	—
	S:Se 605:1	1.20	0.021	0.175	12.5
	2 μg Se/cm^3	0.30	0.433	0.072	64.3

The results of studies concerning the degree of selenium utilization by the yeasts in propagator cultures containing no sulfur but only 1, 2 and 3 μg Se/cm^3 substrate are presented in Table 2. It was found that at the concentration of 1 μg Se/cm^3 the selenium was almost completely consumed by both *C. tropicalis* and *S. cerevisiae*, whereas in the presence of 2 μg Se/cm^3 selenium utilization was 50-65%, and at 3 μg Se/cm^3 the figure stood at 40%. The consumed selenium was not completely incorporated into the structure of the cells since considerable quantities of this element could be removed from the yeasts by dialysis. The content of selenium unattainable by dialysis and at the same time assayable without prior degradation of yeast cell structure was below 1 mg in dry mass yield (Table 2). The remaining selenium assayed after mineralization of yeasts was termed selenium bounded in inorganic compounds. Its content per 1 g dry yeast mass was the lowest in cultures with 1 μg Se/

Table 2. Selenium balance after 15 h of *Saccharomyces cerevisiae* and *Candida tropicalis* culturing in propagators

Yeast	Medium combination μg Se/cm³	Se content in substrate before culture (mg/6 dm³)	Se content in substrate after culture (mg/6 dm³)	Se loss during dialysis of yeast (mg/dry mass yield)	Inorganic Se content (mg/dry mass yield)	Organic Se content (mg/dry mass yield)	Organic Se Content (mg/g dry mass)
S. cerevisiae	1	6.00	0.76	1.72	0.94	2.58	0.30
	2	11.70	4.10	2.24	0.72	4.64	0.69
	3	17.10	10.28	2.61	0.00	4.21	0.93
C. tropicalis	1	5.40	0.07	2.66	0.47	2.20	0.36
	2	11.60	5.75	2.99	0.31	2.55	0.66
	3	17.35	11.53	3.20	0.35	2.27	0.89

/cm³ and highest in cultures containing 3 μg Se/cm³ substrate. The per cent content of selenium bounded in inorganic compounds, calculated in proportion to selenium introduced into the substrate, decreased with the increase of selenium concentration in the medium.

Among the applied combinations of propagator cultures only one medium — the control — contained sulfur compounds; all the other media contained no sulfur. The SO_4 ion traces introduced with the reagents did not give a positive color reaction with p-aminodimethylaniline at this concentration. However, fairly large amounts of this element were found in yeasts from cultures containing no sulfur source (Table 3). Despite triple passaging of germinal yeasts in media without sulfur, 9.99 mg of sulfur in 5.98 g dry mass of *S. cerevisiae*, and 7.79 mg of sulfur in 3.64 dry mass of *C. tropicalis* were introduced into the culture environment. An increase in the number of passages had an adverse effect on the volume of germinal yeast biomass yield. After assaying the sulfur content in dry yeast mass after 15 h of culture in propagators, the same amount of sulfur as the one introduced with the germinal yeasts was found in the yield.

The demonstration of the inhibitory effect of selenium on the growth of *S. cerevisiae* and *C. tropicalis* already at the concentration of 1 μg Se/cm³ contradicts to some extent the results of studies by Blauth-Opieńska and Iwanowski [9] and Tuve and Williams [14]. The former researchers demonstrated that concentrations in excess of 10^{-4} M Na_2SeO_3, which corresponds to 7.9 μg Se/cm³, clearly inhibited the growth of the bacteria *Escherichia coli*, whereas the latter authors found that selenium concentrations below 10 μg Se/cm³ did not affect the growth of *E. coli*. The discrepancy of results is probably due to a different reaction to selenium of *E. coli* cells and the cells of the studied yeasts, or to different methods of culturing.

The experiments with media containing different proportions of sulfur and selenium were meant to check whether selenium can indeed replace sulfur in compounds forming cell structures. A selenium addition to each of the substrates manifested itself with a strong inhibition of yeast growth, although several reports [1, 3, 11, 14, 16] indicate that this element may compete with sulfur in syntheses of all the compounds formed by sulfur in microorganism cells.

The obtained results justify the conclusion that selenium contained in inorganic compounds did not reduce the demand of *S. cerevisiae* and *C. tropicalis* for sulfur. This observation is in agreement with the results of Tuve and Williams [14] but contradicts the findings of Cowie and Cohen [3] who claimed that selenium compounds reduced the demand for sulfur in *E. coli*. The results obtained in this study indicate that sulfate reduced the toxicity of selenium compounds but did not prevent it entirely. The inhibitory effect of selenium increased with the decrease of

Table 3. Sulfur balance in the yeasts *Saccharomyces cerevisiae* and *Candida tropicalis*

Yeast	Medium combination	S content in medium before culture (g/6 dm³)	S content in medium after culture (g/6 dm³)	S content in yeasts (mg/dry mass yield)	S loss due to yeast dialysis (g)	S introduced with inoculum (mg)
S. cerevisiae	Control	7.62	5.52	154.04	1.94	9.99
	Medium without S and Se	—	—	9.80	—	9.99
	1 μg Se/cm³	—	—	10.15	—	9.99
	2 μg Se/cm³	—	—	9.80	—	9.99
	3 μg Se/cm³	—	—	9.65	—	9.99
C. tropicalis	Control	7.20	4.80	101.43	2.30	7.79
	Medium without S and Se	—	—	7.40	—	7.79
	1 μg Se/cm³	—	—	7.76	—	7.79
	2 μg Se/cm³	—	—	7.60	—	7.79
	3 μg Se/cm³	—	—	6.91	—	7.79

the sulfur: selenite ratio. The studies of Weissman and Trelease [16] show that sulfate did indeed reduce toxicity only in the case of low concentrations of selenites, without affecting it at higher selenite concentrations.

The consumption of selenite by yeast cells depended on the presence of sulfur in the medium, which determined the growth of biomass. The greater the biomass increment — the more selenium contained in the substrate was utilized by the yeasts. The presence in the studied yeasts of selenium resisting dialysis and failing to produce a direct reaction with 3.3'-diaminobenzidine indicates that this process was not one of physical sorption but that selenium was incorporated into organic compounds.

CONCLUSSION

1. The magnitude of sodium selenite doses toxic for yeasts depended on the sulfur content in the substrate and on yeast genus.
2. The yeast *Candida tropicalis* was more susceptible to the toxic effects of selenium than *Saccharomyces cerevisiae.*
3. Selenium presence in the medium did not reduce the yeasts' demand for sulfate ions.

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WPŁYW SELENU NA WZROST DROŻDŻY *SACCHAROMYCES CEREVISIAE* I *CANDIDA TROPICALIS*

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Streszczenie

Przebadano wpływ selenu na wzrost drożdży *Saccharomyces cerevisiae* i *Candida tropicalis*. Hodowle prowadzono początkowo na wytrząsarce rotacyjnej, a następnie w propagatorach o poj. 10 dm^3. Drożdże trzykrotnie pasażowane na pożywce pozbawionej źródła siarki i selenu wprowadzano do podłoży zawierających związki siarki (hodowle kontrolne), o zmiennych proporcjach siarki do selenu, bez źródła siarki i selenu oraz bez związków siarki, ale o różnych stężeniach seleninu sodu.

Na podstawie uzyskanych wyników stwierdzono, że każdy stosowany dodatek selenu (1, 2, 4, 6 i 10 $\mu g/cm^3$) do pożywki nie zawierającej źródła siarki hamował proces rozmnażania się komórek drożdży w stopniu większym niż podłoże nie zawierające siarki i selenu (rys. 1, 2 i 3). W obu przypadkach nastąpiło wyraźne zmniejszenie liczby komórek w stosunku do kombinacji kontrolnych. W podłożach zawierających różne stężenia siarki, a jednakowe stężenie selenu (2 μg Se/cm^3) malał przyrost liczby komórek drożdży wraz z obniżeniem zawartości siarki. Wobec tego obecność nieorganicznych związków selenu w pożywce nie zmniejszała zapotrzebowania drożdży na jony siarczanowe. Wielkość toksycznych dawek seleninu sodu zależała również od rodzaju drożdży. Drożdże *Candida tropicalis* były bardziej wrażliwe na toksyczne oddziaływanie selenu niż drożdże *Sacch. cerevisiae*.

Wyniki dotyczące stopnia wykorzystania selenu przez drożdże, wykazały, iż 40-60% selenu zatrzymanego przez drożdże zostało związane w struktury komórkowe, ponieważ nie można go było usunąć przez dializę, a obecność tych ilości selenu wykazano przez analizę chemiczną po uprzedniej mineralizacji dializowanej biomasy (tab. 2).