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EXPERIMENTAL ARTICLES

Selenium Tolerance of Yeasts

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Abstract—Selenium tolerance of yeasts widely varies: the growth of some yeasts can be inhibited by a selenium concentration as low as 10^{-4} M, whereas others can grow in the presence of 10^{-1} M selenium. Homogeneous yeast taxa are characterized by a certain level of selenium tolerance, and heterogeneous taxa show a variable level of tolerance to selenium. In general, ascomycetous yeasts are more tolerant to selenium than basidiomycetous yeasts. Among the ascomycetous yeasts, the genera *Dekkera* and *Schizosaccharomyces* exhibited the lowest and the species *Candida maltosa*, *Hanseniaspora valbyensis*, *Kluyveromyces marxianus*, and *Yarrowia lipolytica* the highest tolerance to selenium. Among the basidiomycetous yeasts, the genera *Bullera*, *Cryptococcus* and *Holtermannia* showed the lowest and the species *Cryptococcus curvatus*, *Cr. humicola*, and *Trichosporon* spp. the highest tolerance to selenium. The selenium tolerance of yeasts depends on the composition of the growth medium, in particular, on the presence of sulfate, sulfur-containing amino acids, and glutamine in the medium.

Key words: selenium, yeasts, tolerance.

Selenium is an essential chemical element for living organisms, since it is a constituent of many enzymes. The presence of selenium in diets prevents the development of cancer tumors [1]. In some regions, the “selenium yeast” *Saccharomyces cerevisiae* is used to compensate for the deficiency of this element in human and animal diets [2]. At the same time, elevated levels of selenium may be toxic to organisms. The toxicity of selenium can be explained by the fact that this element is an analogue of sulfur. Accordingly, when selenium is present in great amounts, it incorporates into sulfur-containing amino acids and then in proteins, changing their conformation and functional activity [3].

In spite of the wide use of the selenium yeast, the effect of selenium on yeasts has not yet been studied in depth. The aim of the present work was to investigate selenium tolerance in a diversity of yeasts.

MATERIALS AND METHODS

The yeast strains studied in this work (404 strains of 181 species belonging to 40 genera) were either obtained from the All-Russia Collection of Microorganisms (VKM) or were isolated by us during the microbiological survey of the Prioksko-Terrasnyi Zapovednik (Oka Terrace Nature Reserve) [4]. The tolerance of yeasts to selenium was tested by growing them at 25°C in a liquid medium containing (g/l) glucose, 10.0; peptone, 5.0; yeast extract, 1.0; agar, 20.0; and Na_2SeO_4 at concentrations varying tenfold from 10^{-5} to 10^{-1} M. This medium was inoculated with yeast cells grown on wort agar for 2–3 days. The effect of glutamine (5 g/l), $(NH_4)_2SO_4$ (3 g/l), and the sulfur-containing amino acids methionine and cysteine (3 g/l) on the selenium tolerance of yeasts was studied by adding these compounds at indicated concentrations to an agar synthetic medium lacking Na_2SeO_4 and amino acids. Cell suspensions used for inoculation contained 10^6 cells/ml. The control cultures were grown in the same media as the experimental cultures, but without Na_2SeO_4.

Table 1. Yeast species and the number of strains whose growth is partially suppressed by 10^{-5} M Na_2SeO_4 and completely inhibited by 10^{-4} M Na_2SeO_4

Bullera alba (5)	*Cr. phenolicus* (1)
B. armeniaca (1)	*Cr. terreus* (7)
B. pseudoalba (1)	*Cr. terricola* (1)
Candida silvae (1)	*Cr. vishniacii* (2)
Cryptococcus chernovii (1)	*Dekkera anomala* (2)
Cr. dimennae (1)	*Holtermannia corniformis* (2)
Cr. flavus (1)	*Pichia norvegensis* (1)
Cr. fuscescens (1)	*Rhodotorula yakutica* (1)
Cr. hungaricus (1)	*Schizosaccharomyces pombe* (2)
Cr. marinus (1)	

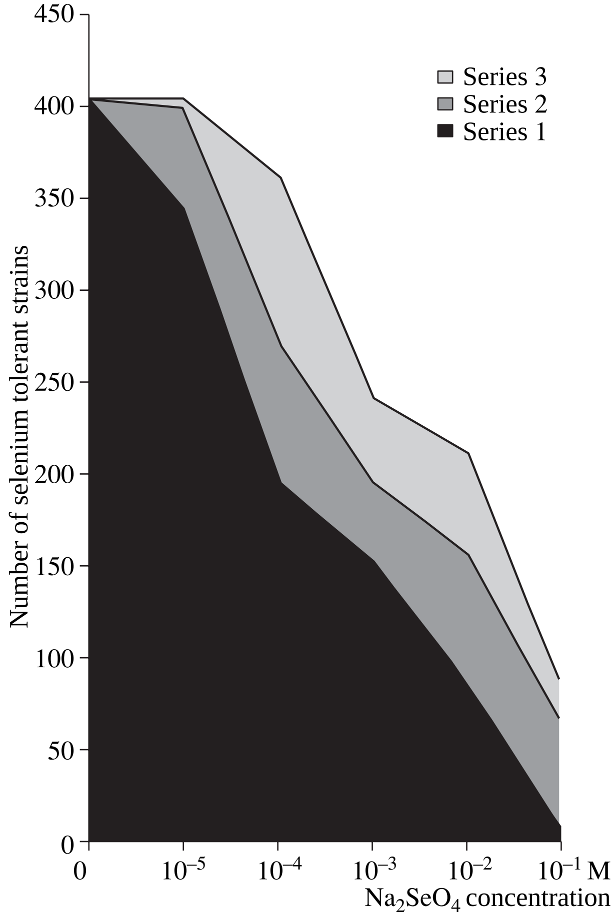

Fig. 1. The distribution of yeast strains according to the ability to grow in the glucose–peptone medium in the presence of different concentrations of Na_2SeO_4. Series 1, good growth; series 2, slow latent growth; and series 3, poor growth.

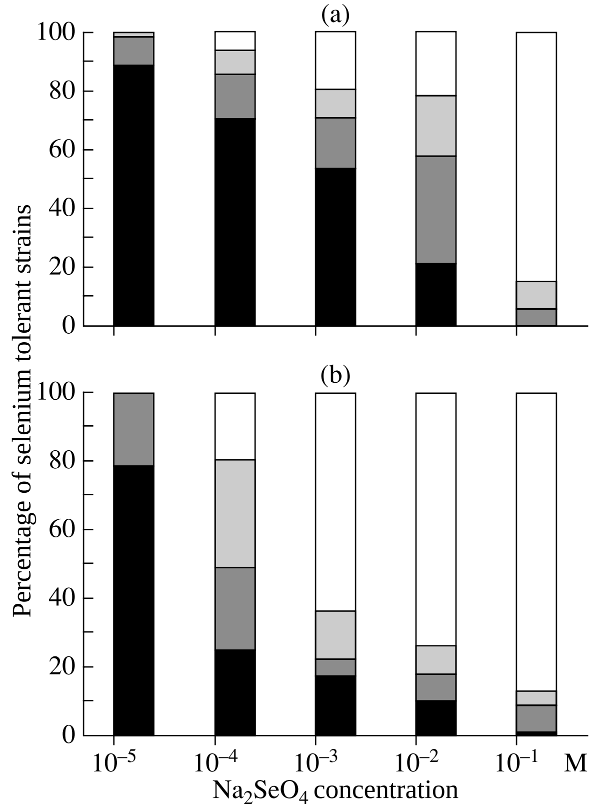

Fig. 2. The distribution of (a) ascomycetous and (b) basidiomycetous yeasts according to the ability to grow in the glucose–peptone medium in the presence of different concentrations of Na_2SeO_4. The dark portions of the bars indicate the percentage of yeast species capable of growing at the particular concentration of Na_2SeO_4.

μMRESULTS

Na_2SeO_4 at a concentration of 10^{-5} M did not influence the growth of almost all of the yeast strains under study on glucose–peptone agar, except for some species of the genus *Cryptococcus*, whose growth was somewhat suppressed at this selenium concentration and was partially or completely inhibited at a selenium concentration of 10^{-4} M (Tables 1 and 3). Most of the yeast strains could grow at the last selenium concentration, but only half of them grew poorly at a selenium concentration of 10^{-3} M. Na_2SeO_4 at a concentration of 10^{-2} M inhibited the growth of most of the yeast strains (Figs. 1 and 2), and only some strains could grow at a selenium concentration of 10^{-1}M (Table 2). In the last case, the growth was poor, and the yeasts produced pink- to red-colored colonies, whereas normally (i.e., in the absence of Na_2SeO_4) they are colorless. In the course of further incubation, colorless secondary colonies appeared on the surface of the red colonies.

Among the ascomycetous yeasts studied, the genera *Citeromyces*, *Clavispora*, *Dekkera*, and *Schizosaccharomyces* showed the lowest tolerance to selenium (the members of these genera could grow at selenium concentrations not exceeding 10^{-4} M). At the same time, almost all species of the genera *Aciculoconidium*, *Hanseniaspora*, *Kazachstania*, *Kluyveromyces*, *Mastigomyces*, *Nadsonia*, *Saccharomyces*, *Saccharomycoides*, *Schizoblastosporion*, *Wickerhamia*, and *Yarrowia* were able to grow at a selenium concentration of 10^{-2} M. Among the basidiomycetous yeasts studied, only two cryptococci, *Cr. curvatus* and *Cr. humicola*, and some species of the genus *Trichosporon* exhibited high selenium tolerance (Table 2), whereas the other basidiomycetous yeasts studied could grow at selenium concentrations not exceeding 10^{-4} M.

Some yeast genera were found to be heterogeneous with respect to selenium tolerance. For instance, the maximal concentrations of selenium appropriate for the growth of *Candida* spp. varied from 10^{-5} to 10^{-1} M. Similarly, some yeast species also exhibited heterogeneity with respect to selenium tolerance (Table 3).

Table 2. Yeast species and the number of strains which are able to grow in the presence of 10^{-1} M Na_2SeO_4

Candida maltosa (3)	*Tr. coremiiforme* (1)
Cryptococcus curvatus (3)	*Tr. cutaneum* (6)
Cr. humicola (4)	*Tr. dulcitum* (1)
Hanseniaspora valbyensis (2)	*Tr. gracile* (1)
Kluyveromyces marxianus (23)	*Tr. moniliiforme* (1)
Trichosporon aquatile (1)	*Yarrowia lipolytica* (19)
Tr. brassicae (1)	

Sulfates in the growth medium prevented the inhibitory effect of selenium on yeasts. For instance, in the presence of $(NH_4)_2SO_4$ and 10^{-3} M Na_2SeO_4 in the medium, good growth was observed for members of the genera *Bullera, Cryptococcus, Cystofilobasidium, Dekkera, Erythrobasidium, Fibulobasidium, Filobasidium, Holtermannia, Pseudozyma, Rhodotorula, Schizosaccharomyces*, and *Tsuchiyaea*, which grew poorly, if at all, in the presence of 10^{-4} M selenium when ammonium sulfate was absent in the medium. Of interest is the fact that ammonium sulfate did not exert any beneficial effect on the highly selenium tolerant yeasts (Table 2), whereas the sulfur-containing amino acids methionine and cysteine enhanced the growth of these yeasts in the presence of 10^{-1} M Na_2SeO_4. Glutamine also increased the selenium tolerance of yeasts about tenfold.

DISCUSSION

As can be seen from the data presented, almost all of the yeasts studied grew well in the presence of 10^{-5} M Na_2SeO_4, and only some of them were inhibited by 10^{-4} M Na_2SeO_4. However, as the selenium concentration was raised further, the number of yeasts capable of growth at a particular selenium concentration steeply declined (Fig. 1).

Table 3. Yeast taxa which are heterogeneous with respect to selenium tolerance

Species (synonyms) and strains	Na_2SeO_4 concentration in the medium			
	10^{-4}	10^{-3}	10^{-2}	10^{-1}
Candida rugosa BKM Y-1511 (*C. rugosa* var. *elegans*, T)	s	p	–	–
67T	+	+	s	p
Cryptococcus albidus BKM Y-714 (*Torula albida*, T),				
751 (*Torulopsis nadaensis*, T), 1983, 1984, 2222	p	–	–	–
1531 (*Cr. genitalis*, A), 1539 (*Cr. albidus* var. *ovalis*, T), 1954, 1955, 2561	s	p	–	–
1646 (*Cr. albidus* var. *kuetzingii*, T), 2223T	+	p	p	–
Cr. laurentii BKM Y-720 (*Torulopsis carnescens*, T),				
1291 (*Rhodotorula peneaus*, T), 1594	–	–	–	-
328 (*Rh. aurea*, T), 1032, 1595 (*Cr. laurentii* var. *flavescens*, T), 1987, 2244	p	–	–	–
1627, 1628, 1665í	s	p	p	–
Kluyveromyces thermotolerans BKM Y-894T	–	–	–	–
533 (*K. veronae*, T), 534, 2317	+	s	p	–
Rhodotorula mucilaginosa BKM Y-83 (*Cr. ludwigii*),				
755 (*Torulopsis nitritophila*, T), 1128, 1152, 1308, 1324, 1325, 2058, 2286, 2297	p	–	–	–
80 (*Cr. corallinus*, T), 691 (*Torula aclotiana*, T),				
1117 (*Cr. pararoseus*, T), 1119 (*Cr. rubrorugosus*, T),				
1123, 1320, 1323, 2283 (*Rh. matritensis*, T)	s	p	–	–
7 (*B. carbonei*, T), 17 (*Blastodendrion simplex*, T),				
87 (C*r. radiatus*, T), 253 (*Mycotorula cisnerosi*, T),				
339T, 341 (*Rh. rubra*, T), 343 (*Rh. rubra* var. *longa*, T), 344 (*Torulopsis sanniei*, T), 718 (*Torulopsis biourgei*, T), 747 (*Torulopsis mannitica*, T), 1127, 2650 (*Rh. grinbergsii*, T)	+	s	p	–
Trichosporon porosum BKM Y-3T	p	–	–	-
2866, 2867	+	c	p	–

Note: T denotes "type strain." "+," "s," "p," and "–" stand for "good growth," "slow growth," ""poor growth," and "no growth," respectively.

In general, ascomycetous yeasts are found to be more selenium tolerant than basidiomycetous yeasts (compare Figs. 2a and 2b). The only genus of ascomycetous yeasts that is tolerant to high selenium concentrations is *Trichosporon.* This observation does not contradict the fact that the list of highly selenium tolerant yeasts (Table 2) includes two cryptococci and no *Tr. pullulans*, since it is known that yeast taxa are extremely heterogeneous [5]. In particular, the results of rDNA sequencing suggest that the species *Cr. curvatus* and *Cr. humicola* belong to the order *Trichosporonales*, whereas the species *Tr. pullulans* belongs to a different order, *Cystofilobasidiales* [6, 7]. Undoubtedly, these three species need reclassification, as a result of which *Cr. curvatus* and *Cr. humicola* may appear in the genus *Trichosporon* Behrend, and *Tr. pullulans*, in the genus *Tausonia* Babjeva.

The high tolerance of the genus *Trichosporon* to selenium is a unique feature among basidiomycetous yeasts and, hence, selenium tolerance can be used for taxonomic purposes and for the selective isolation of this genus from natural and clinical sources. Similarly, media with increased concentrations of Na_2SeO_4 can be used for the selective isolation of some ascomycetous yeasts, such as those of the genus *Yarrowia* (Table 2).

Noteworthy is the fact that heterogeneous yeast taxa also exhibit heterogeneity with respect to selenium tolerance. Taxonomic heterogeneity is especially pronounced in the genus *Candida* and in the yeast species *Cr. albidus* [8], *Cr. laurentii* [9], and *Rh. mucilaginosa* [10]. These species, as well as some others, exhibit high heterogeneity of their strains with respect to selenium tolerance (Table 3) and, hence, their synonymy should be approved by modern methods. Type strains, which are the oldest isolates of particular species, show decreased tolerance to selenium. Consequently, the possibility cannot be excluded that their low selenium tolerance resulted from their long-term maintenance under laboratory conditions.

The degree of selenium tolerance of a yeast is determined by its ability to distinguish selenium and sulfur in the metabolic processes in which these chemical elements are involved. The anions SeO_4 and SO_4 compete with one another already at the stage of their uptake by a cell, which is accomplished by the same transport system [11]. The competition between these anions for mutual permeases may explain the beneficial effect of $(NH_4)_2SO_4$ on the yeasts with low or medium tolerance to selenium. At the same time, this mechanism of selenium detoxication by ammonium sulfate probably does not work in the yeasts with high tolerance to selenium. These yeasts may accomplish other detoxication mechanisms, such as the reduction of the Se ion to elemental Se [12, 13], as is evident from the formation of pink- to red-colored colonies on agar media with a high content of Na_2SeO_4. In this case, the synthesis of sulfur-containing amino acids remains suppressed, as revealed by the growth-stimulating effect of the sulfur-containing amino acids added to the medium. Another possible mechanism of selenium detoxication is its incorporation into the amino acids γ-glutamylmethylselenocysteine and γ-glutamylmethylselenocystathionine, which are not used by cells for protein synthesis [3]. The fact that this mechanism does function in yeasts is evident from the detoxicating effect of high concentrations of glutamine in the growth medium.

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