ISSN 0026-2617, Microbiology, 2015, Vol. 84, No. 3, pp. 442–448. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © A.M. Glushakova, A.V. Kachalkin, T.M. Zheltikova, I.Yu. Chernov, 2015, published in Mikrobiologiya, 2015, Vol. 84, No. 3, pp. 379–385.

EXPERIMENTAL ARTICLES

Resistance of Various Yeast Ecological Groups to Prolonged Storage in Dry State

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Received July 25, 2014

Abstract—Resistance of 14 yeast species belonging to different ecological groups to extensive storage in a dried state was investigated. Pedobiotic yeasts isolated mainly from the soils of humid areas (*Cryptococcus podzolicus, Cr. terricola*, and *Lipomyces starkeyi*) were the least resistant. The yeasts associated with the nectar of entomophilous plants (*Metschnikowia reukaufii* and *Candida bombi*) also exhibited low resistance to drying. Complete death of these species occurred during the first month of storage. Eurybiotic species from various environments (*Cryptococcus magnus, Cryptococcus victoriae, Debaryomyces hansenii*, and *Cryptococcus wieringae*) were somewhat more resistant. Pigmented plant-associated yeasts (*Rhodotorula mucilaginosa* and *Sporobolomyces roseus*), as well as the pathogenic or opportunistic *Candida* strains (*C. albicans* and *C. parapsilosis*), were the most resistant to drying. Thus, occurrence of yeasts in natural habitats is closely associated with their ability to survive prolonged drying.

Keywords: yeasts, resistance, drying, relative air humidity, *Candida albicans*

DOI: 10.1134/S0026261715030066

The problem of the effects of water activity and relative air humidity on yeast growth has many aspects. Availability of water, which is usually characterized by the value of water activity (a_w), is the most important factor limiting yeast growth. A low level of a_w, which is characterized by low water content, water crystallization, and increased concentrations of osmotically active substances, is typical of a number of natural and artificial cenoses [1, 2]. Information on the a_w values sufficient for development of various yeast species is required in many fields, e.g., for storage and transportation of foodstuffs, protection of cultural heritage and items of art, prognosis for the yeast growth and propagation under natural conditions and in living quarters, and development of recommendations for the microclimate support in living spaces, as well as for the planning of preventive measures in the biocontrol practice [3].

In mycelial fungi and yeasts, low a_w values cause retardation of spore germination, elongation of the periods from spore germination to development of mycelium and spores, and a decrease in the rate of colony radial growth. However, various yeasts and micromycetes are characterized by different sensitivity to low a_w values [4–7]. Xerophilic and xerotolerant yeasts realize the strategy of cell protection by accumulation of osmotically active substances, mainly glycerol, which is either consumed from the substrate or synthesized *de novo* [8, 9].

Water activity is the most important factor limiting the yeast propagation in nature. Most yeast species are unable to grow at a_w below 0.70–0.61, which is characteristic of the solutions of 55–65% glucose or 15–25% NaCl [10]. The most osmotolerant yeasts, which are capable of growth in rather concentrated sugar solutions, were revealed among the genera *Saccharomyces*, *Zygosaccharomyces*, and *Schizosaccharomyces*. Many strains of the eurybiotic species *Debaryomyces hansenii* are halotolerant and often occur in various salted foods and canned meat products.

The capability of yeasts for prolonged storage in a dried state determines their success in occupying natural ecotopes. Yeasts were often isolated from the habitats with permanently low humidity, such as dry grasses, dust, and desert soils. Moreover, the maximum number of epiphytic yeasts is usually revealed in dry aerial parts of plants rather than in actively growing ones. For instance, we found yeasts belonging mainly to the species *Cryptococcus albidus* (up to 10^6 CFU/g) in dry parts of plants and fallen leaves from the Kyzyl Kum and Karakum deserts [11].

Yeast species are different in their resistance to prolonged storage in a dried state. The factors responsible for their resistance to drying have been insufficiently studied; one of them is considered to be ability of the cell to produce polysaccharide capsules [12, 13]. In

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particular, capsular forms of widespread yeasts *Cryptococcus albidus* were shown to be more resistant to drying than the capsule-free variants [14].

Numerous studies on the ability of various yeast species to endure prolonged drying deal mainly with the problem of storage and preservation of lyophilized yeast strains in the culture collections. However, in the last years, wide application of cryoconservation methods decreased interest in this problem. Ecological aspects of the yeast resistance to prolonged drying have not been studied.

The goal of the present work was to compare the resistance of different yeast species to prolonged drying in relation to their ecological features and natural habitats.

MATERIALS AND METHODS

The ability of yeasts to grow after prolonged storage under starvation and low relative air humidity was investigated. The study was performed with yeast species routinely found in natural plant and soil substrates in central Russia; the patterns of their occurrence were well known and statistically confirmed by perennial observations [15]. We isolated yeast strains from various natural substrates in 2010–2012 and identified them by analysis of the nucleotide sequences of the D1/D2 region of 26S (LSU) rDNA. All strains designated as KBP were maintained in the Yeast Collection of the Department of Soil Biology, Faculty of Soil Science, Moscow State University; strains of the facultatively pathogenic species *Candida albicans* and *C. parapsilosis* (designated as NIIVS) were stored in the Collection of the Mechnikov Institute for Vaccines and Sera, Russian Academy of Medical Sciences. Each species was represented by 2–4 strains; in all, 35 strains belonging to 13 species were studied (table). The species were chosen in such a way that they represented various living forms associated with different types of ecotopes. Typical pedobiotic yeasts (*Lipomyces starkeyi*, *Cryptococcus terricola*, and *Cr. podzolicus*) were isolated from different soil horizons; eurybiontic species (*Cr. magnus*, *Cr. victoriae*, *Cr. wieringae*, and *Debaryomyces hansenii*) occurred in almost the same numbers in different natural ecotopes; typical phytobionts (*Rhodotorula mucilaginosa* and *Sporobolomyces roseus*) were mainly associated with various aerial parts of plants; "nectarous" yeasts (*Metschnikowia reukaufii* and *Candida bombi*) were typical inhabitants of the nectar of entomophilous plants; facultatively pathogenic species (*C. albicans* and *C. parapsilosis*) occurring in various environmental substrates are mainly known as representatives of the normal human microflora capable of causing candidiasis. None of the studied strains formed any dormant forms (ascospores, chlamydospores, or teliospores); the perfect species were represented by anamorphic yeasts.

The strains were grown on glucose-peptone-yeasts extract agar (GPYA) for 7 days at 25°C. Microscopic

The studied yeast strains

Species	Strains
Candida albicans	NIIVS 201, NIIVS 561
Candida bombi	KBP 3935, KBP 3936, KBP 3937, KBP 4092
Candida parapsilosis	NIIVS 705, NIIVS 967, NIIVS 1204
Cryptococcus magnus	KBP 4129, KBP 4489, KBP 4490
Cryptococcus podzolicus	KBP 6, KBP 4077, KBP 4198
Cryptococcus terricola	KBP 3960, KBP 4051, KBP 4068
Cryptococcus victoriae	KBP 3862, KBP 4014, KBP 4015, KBP 4273
Cryptococcus wieringae	KBP 3928, KBP 3963
Debaryomyces hansenii	KBP 4033, KBP 4058, KBP 4059
Lipomyces starkeyi	KBP 105, KBP 2559
Metschnikowia reukaufii	KBP 4036, KBP 4163
Rhodotorula mucilaginosa	KBP 3859, KBP 4169, KBP 4170
Sporobolomyces roseus	KBP 2555, KBP 4286

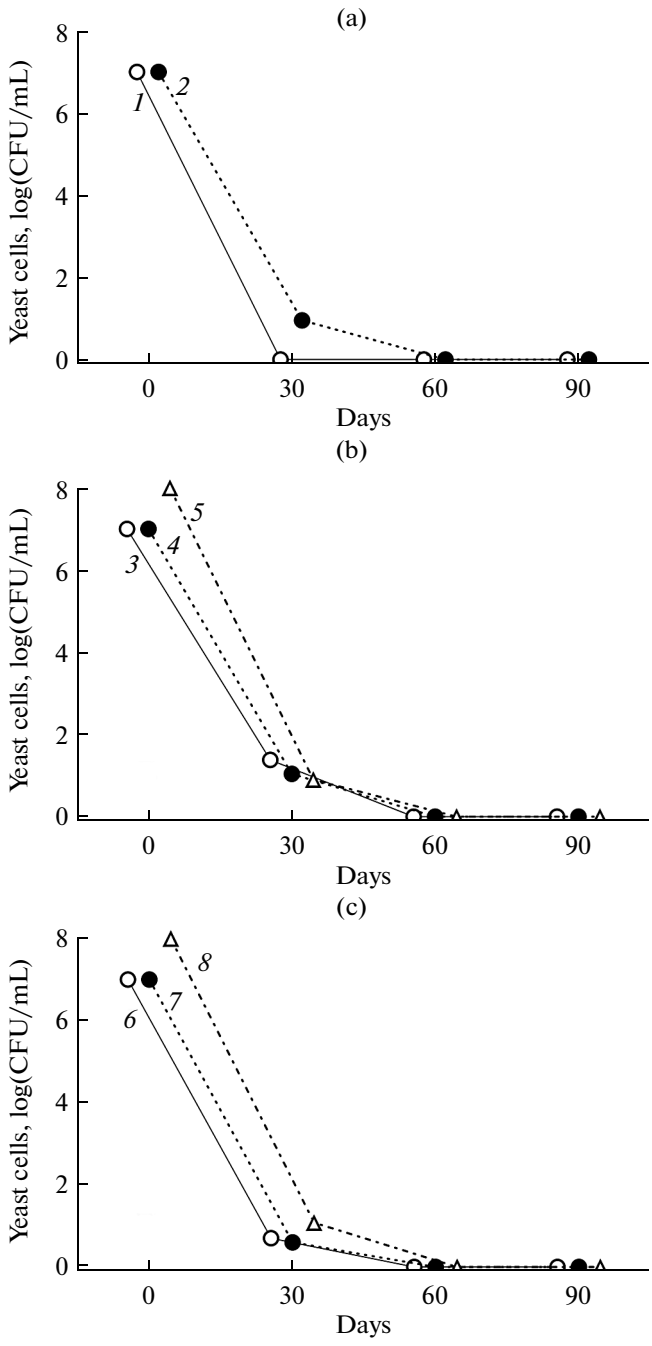

Fig. 1. Changes in the number of pedobiotic yeasts in the course of 90-day storage in a dried state. Species: *L. starkeyi* (a), *Cr. terricola* (b), and *Cr. podzolicus* (c). Strains: KBP 105 (*1*), KBP 2559 (*2*), KBP 3960 (*3*), KBP 4051(*4*), and KBP 4068 (*5*).

examination revealed a great number of budding cells in all of the studied strains, which was indicative of active yeast growth. Cell suspensions of each strain in water (100 μL) were applied onto sterile paper filters of 1 cm^2 (20 filters for each strain), dried in a laminar flow cabinet, and then placed into sterile petri dishes (five filters per a dish), which were incubated in a sterile box at 25°C for 90 days. Relative air humidity and temperature were controlled with a TK-5.06 thermometer with a function of measuring the relative humidity. Relative air humidity in the boxes was below 40% in the period from the 30th day to the end of experiment.

The dried filters were taken in 30, 60, and 90 days of storage, transferred into sterile Eppendorf tubes with 1 mL of sterile water, and homogenized on a vibration mixer (MultiReax, Heidolph, Germany) for 60–75 min. The obtained suspension (100 μL) was plated onto GPYA (five replicates for each filter) and incubated at 25°C. In the control, inoculation was

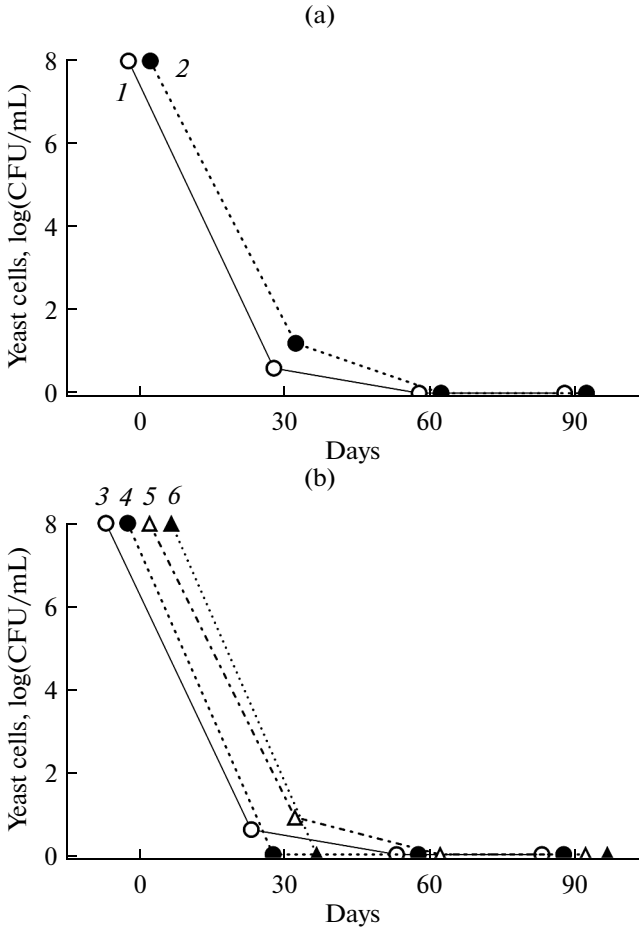

Fig. 2. Changes in the number of "nectarous" yeasts in the course of 90-day storage in a dried state. Species: *M. reukaufii* (a) and *C. bombi* (b). Strains: KBP 4036 (*1*), KBP 4163 (*2*), KBP 3935 (*3*), KBP 3936 (*4*), KBP 3937 (*5*), and KBP 4092 (*6*).

performed immediately after application of the initial cell suspension onto the filters. The average number of colony-forming units (CFU) for each strain was calculated per 1 mL of the homogenized suspension. The obtained results were statistically processed. On the figures, one unit was added to the logarithm of the data; therefore the minimal logarithm of the cell number was 0.

RESULTS AND DISCUSSION

Survival rates of the yeast species belonging to various ecological groups in the course of storage in a dried state were found to differ considerably. Some species were unable to grow after one month in storage, while in the others, the cell number decreased gradually in the course of experiment. The viability value (CFU/mL) exhibited less intraspecific variability between the strains compared to interspecific differences. Importantly, a correlation was found between resistance to storage in a dried state and yeast ecotopes. Pedobiotic yeasts represented by species *L. starkeyi*, *Cr. podzolicus*, and *Cr. terricola* were the least resistant to drying; they completely lost viability within the first month of storage (Fig. 1), which is in accordance with their occurrence in nature. These species usually inhabit the upper horizons of boreal soils in a humid climate with relatively constant humidity. It is noteworthy that all these species form thick polysaccharide capsules. Thus, our study did not confirm the assumed protective role of capsules against cell drying.

Ascomycetous yeasts *M. reukaufii* and *C. bombi* also completely died after a 2-month storage in a dried state (Fig. 2). These species are found mainly in the nectar of entomophilous plants, with *M. reukaufii* amounting for up to half of the total yeast number in the period of plant blossoming. These species are also closely associated with pollinating insects, which serve as efficient yeast carriers. It should be noted that nectarous yeasts are not revealed in dry plant residues in noticeable amounts [16]. There is information that these yeasts survive unfavorable periods in the nests of insects, in particular, in honeycombs of bees and bumblebees [15].

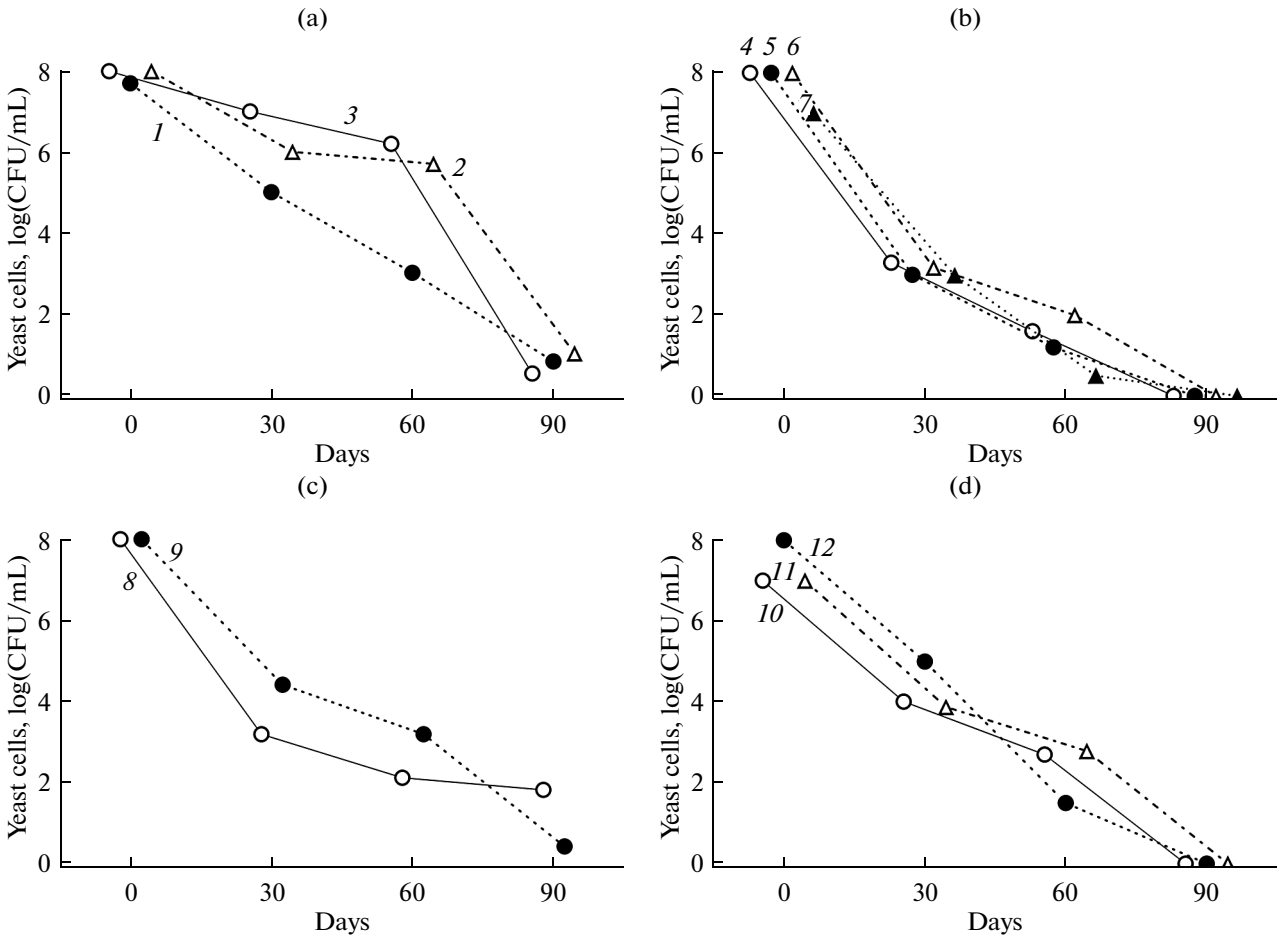

Fig. 3. Changes in the number of eurybiontic yeasts in the course of 90-day storage in a dried state. Species: *Cr. magnus* (a), *Cr. victoriae* (b), *Cr. wieringae* (c), and *D. hansenii* (d). Strains: KBP 4129 (*1*), KBP 4489 (*2*), KBP 4490 (*3*), KBP 3862 (*4*), KBP 4014 (*5*), KBP 4015 (*6*), KBP 4273 (*7*), KBP 3928 (*8*), KBP 3963 (*9*), KBP 4033 (*10*), KBP 4058 (*11*), and KBP 4059 (*12*).

Yeast species *Cr. magnus*, *Cr. victoriae*, *Cr. wieringae*, and *D. hansenii* were more resistant to drying (Fig. 3). These basidiomycetous eurybiotic species are often revealed in ecotopes of various types [16]. Especially active growth of these yeasts occurs in the phyllosphere of trees and grasses and on the tree bark in autumn, in the period of the maximum atmospheric precipitation and high relative air humidity; however, they are also abundant in dry decomposing plant residues in the upper soil horizons. In our experiments these species (especially *Cr. magnus*) exhibited the highest interstrain variability, which is interesting to note. Ascomycetous yeasts *D. hansenii* also belong to eurybiontic species; they occur at almost the same extent in all types of natural ecotopes [15].

Species of pigmented yeasts, such as *Rh. mucilaginosa* and *Sp. roseus*, were the most resistant to drying (Fig. 4). These yeasts are typical phytobionts, inhabiting mainly the living and dead parts of plants [15]. Carotenoid pigments are known to fulfill numerous protective functions under environmental impact, such as antioxidant activity, which appears to provide for the survival of pigmented yeasts during their storage in a dried state.

At the same time, the presence of carotenoids was not the only factor responsible for resistance to drying. It has already been shown that strains of *Cr. albidus* exhibit high survival rates under drying although they contained neither carotenoid pigments nor capsules [14]. The resistance of this species is due to the presence of insulated water in dried cryptococcal cells because of a change in the permeability of intracellular membranes. It should be noted that *Cr. albidus* is practically the only yeast species occurring in the soils of subtropical deserts [11]. The studied species *Cr. magnus* and *Cr. wieringae*, which are phylogenetically related to *Cr. albidus*, also showed rather high resistance to drying.

High viability in a dried state of the ascomycetous yeasts belonging to a group of facultatively pathogenic

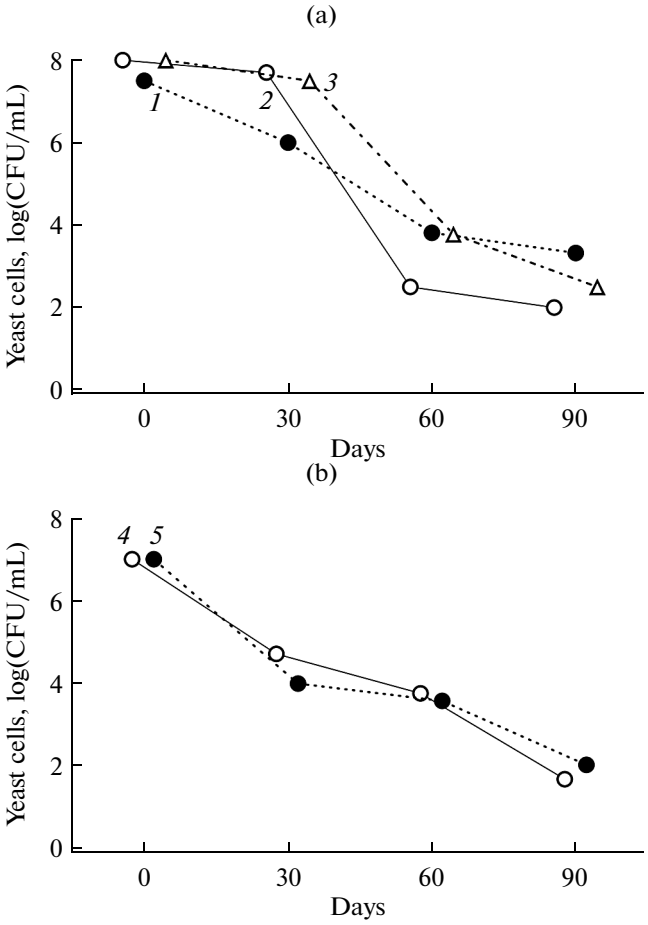

Fig. 4. Changes in the number of pigmented phytobiont yeasts in the course of 90-day storage in a dried state. Species: *Rh. mucilaginosa* (a) and *Sp. roseus* (b). Strains: KBP 3859 (*1*), KBP 4169 (*2*), KBP 4170 (*3*), KBP 2555 (*4*), and KBP 4286 (*5*).

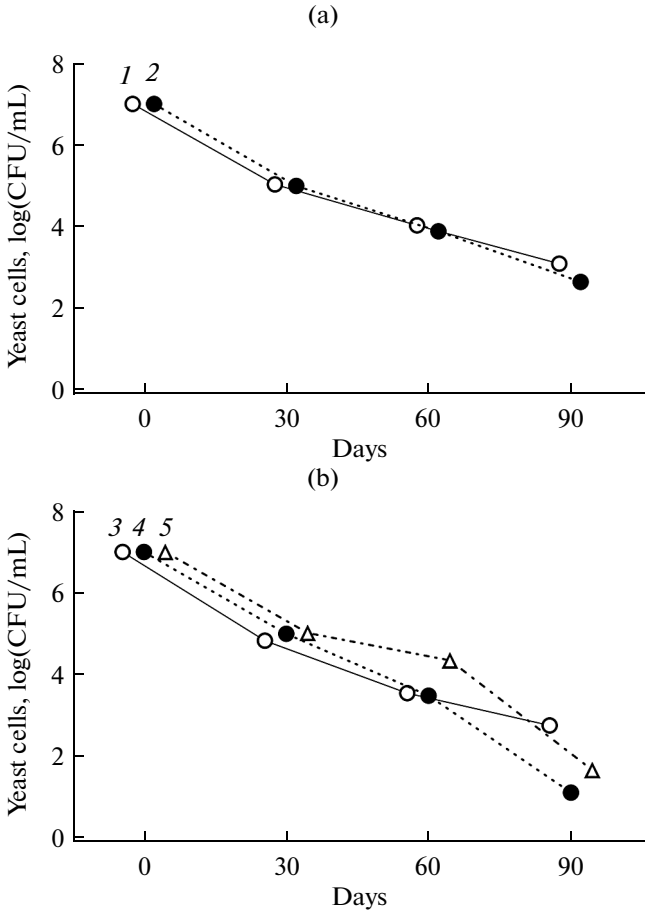

Fig. 5. Changes in the number of pigmented facultatively pathogenic yeasts in the course of 90-day storage in a dried state. Species: *C. albicans* (a) and *C. parapsilosis* (b). Strains: NIIVS 201 (*1*), NIIVS 561 (*2*), NIIVS 705 (*3*), NIIVS 967 (*4*), and NIIVS 1204 (*5*).

species *C. albicans* and *C. parapsilosis* was unexpected (Fig. 5). These yeasts are seldom found in soil and plant residues; as a rule they are a part of the normal human microflora. However, we routinely isolated these yeasts from such substrates with low water activity as the dust of living spaces and the pollen of wind-pollinated plants [17, 18]. The cause of resistance of these species to drying requires further investigations.

Thus, the obtained results indicate that occurrence of yeasts in natural and anthropogenic ecotopes is closely associated with their resistance to prolonged drying. Occurrence of high numbers of yeasts in such substrates as dry plant residues and dust is most probably due to high resistance of these species to storage in a dried state. The yeast viability is apparently determined by the antioxidant properties of carotenoids, as well as by the features of their intracellular structure, which provide for water storage inside the cells. The yeast species possessing these features prevail in protractedly dry habitats because of their capability for survival in inactive state.

ACKNOWLEDGMENTS

Conducting of genetic identification of cultures was supported by the Russian Science Foundation, project no. 14-50-00029.

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Translated by E. Dedyukhina