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EXPERIMENTAL ARTICLES

Alkalitolerant Yeasts from Natural Biotopes

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Abstract—Using a solid nutrient medium containing alkaline buffer (pH 10) and an antibiotic, alkalitolerant yeasts were isolated from samples of soda-rich saline soils (solonchaks) of Armenia (Arazdayan) and the Transbaikal region (the Kungur Steppe). The species diversity of the yeast populations of the tested soda-rich soils was relatively insignificant. They only contained alkalitolerant representatives of asporogenic capsulated yeasts belonging to the species *Cryptococcus laurentii, C. albidus, Rhodotorula glutinis, R. mucilaginosa,* and *Sporobolomyces roseus. C. laurentii* representatives clearly dominated the isolates obtained, their number exceeding that of the other species by two to three orders of magnitude. All of the isolates grew on acidic wort agar, suggesting that they did not include obligate alkaliphiles.

Key words: alkalitolerant yeast, soda-rich solonchaks, alkaliphily.

Alkaliphily has been for a long time considered a peculiarity of a limited group of prokaryotes. It was nonhalophilic bacilli [1] and extremely halophilic, moderately alkaliphilic archaea (natronobacteria) from soda-containing habitats [2] that were investigated in most detail.

However, considerable advances have been made over the last five to six years in the research on the biodiversity of the haloalkaliphilic community of soda lakes, which represent unique natural saline and alkaline habitats. The microbial population of such lakes is highly heterogeneous and consists of prokaryotic representatives of various branches of the phylogenetic tree [3, 4]. Eukaryotic organisms represent minor components of the communities of these habitats and include alkalitolerant microalgae [5] and protozoans.

Soda-containing saline soils are also important natural habitats characterized by high alkalinity. However, few studies on their microbial populations have been conducted up to now. There is some evidence that they contain natronobacteria and haloalkaliphilic *Bacillus* and *Halomonas* [6–8], but no yeasts have yet been detected in these soils.

The goal of this work was to detect and isolate yeast fungi from soda-rich saline soils (solonchaks) and to identify the alkalitolerant strains obtained therefrom.

MATERIALS AND METHODS

Samples of alkaline soils were taken in the Kungur Steppe (Chita oblast, loose solonchak, water extract pH 10.5) and Armenia (Arazdayan, loose solonchak, water extract pH 10–10.5).

One gram of soil was placed into 10 ml of a sterile 1% solution of NaCl. The soil suspension was thereupon treated with a RT-2 tissue microdesintegrator at 3000 rpm for 2 min.

The nutrient medium used for enrichment and isolation contained a mineral base that allowed pH to be maintained at a value no lower than 9.5 [9]: 24 g/l Na_2CO_3, 6 g/l $NaHCO_3$, 6 g/l NaCl, 1 g/l K_2HPO_4, and 1 g/l KNO_3. Upon sterilization, the mineral base was supplemented with 1 ml/l of a trace element solution [10] and 1 ml/l of a 10% $MgSO_4$ solution. Wort (300 ml/l of medium) was added as the carbon and energy source.

A solid alkaline medium was prepared by mixing a separately sterilized mineral base with an equal volume of sterile 4% wort agar at a temperature of 55°C.

Rifampicin (2 mg/ml) was added to the medium to suppress bacterial growth. Apart from the alkaline medium, rifampicin-containing neutral wort agar and acidified wort agar were also used. Inoculated plates were incubated in a refrigerator at 10°C for several months.

Identification of the pure yeast culture obtained was performed by conventional methods [11].

RESULTS

Alkalitolerant yeast from samples of soda-rich solonchaks were isolated after prolonged incubation at 10°C on solid rifampicin-containing alkaline medium. Based on the cultural, morphological, and physiological properties of the isolates obtained, we identified them as representatives of the previously known yeast species *Cryptococcus laurentii* (Kufferath) C.E. Skin-

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Isolation of Yeasts from Alkaline Soil Samples

Region of sampling	Samples	Medium used for isolation and yeast number (cells/g soil)	Yeast species	Growth of isolates on alkaline media: time necessary for the formation of visible colonies
1		Antibiotic-containing alkaline medium		
Kungur Steppe, Chita oblast	Loose solonchak (pH 10.5)	up to 2–3 thousand cells/g	*C. laurentii*	3–4 days
		single cells	*C. albidus*	1 week
		single cells	*Rh. glutinis*	3–4 days
		single cells	*Rh. muckilaginosa*	2 weeks (scarce growth)
2		Antibiotic-containing alkaline medium		
Araxdayan. Ararat Valley, Armenia	Loose solonchak (pH 10.0–10.5)	up to 2–3 thousand cells/g	*C. laurentii*	3–4 days
		single cells	*C. albidus**	3 days to 2 weeks
		Wort agar (pH 5.0)	*Aureobasidium pullulans*	
		Antibiotic-containing neutral wort agar; solitary cells		1 week
		single cells	*C. albidus*	no growth
		single cells	*S. roseus*	3 weeks

* *C. albidus* isolates differed in their growth rate on the surface of alkaline solid medium.

ner (1950), *C. albidus* (Saito) C.E. Skinner (1947b), *Rhodotorula glutinis* (Fresenius) F.C. Harrison (1928), *R. mucilaginosa* (Jorgensen) F.C. Harrison (1928), and *Sporobolomyces roseus* Kluyver a. van Niel (1924).

The capacity to utilize glucosamine was the most important distinction of the alkalitolaterant isolates of *C. laurentii C. albidus* and distinguishes them from previously known representatives of these species. Presumably, glucosamine assimilation is accompanied by NH_3 excretion, which results in an increase in the pH of the medium that is not fatal for the alkalitolerant yeast.

C. laurentii was the predominant species among the isolates obtained. Its population density amounted to 2000–3000 cells per g of soil, whereas the cell numbers of alkalotolerant representatives of other yeast species were insignificant (table). Some of the samples used yielded no isolates at all.

The yeast strains isolated on neutral medium were tested for their capacity to grow under alkaline conditions. This capacity was established in the case of *S. roseus*, while the *C. albidus* strain isolated on neutral wort agar failed to grow on an alkaline medium (table).

Only dark-colored yeasts of the genus *Aureobasidium* were isolated from alkaline soil samples plated onto acidic wort agar. The yeasts grew well after transfer to alkaline agarized medium, although the colony morphology changed because the mycelial developmental phase did not occur. The colonies resembled dry clumps whose color gradually darkened and finally became black.

Interestingly, the pure cultures of the alkalitolerant yeasts that we isolated from alkaline soil samples differed with respect to their capacity to grow on alkaline solid media. The most active isolates belonged to the species *C. laurentii* and *R. glutinis*. They formed visible colonies on the 3rd or the 4th day of cultivation. Alkalitolerant representatives of the species *C. albidus*, *S. roseus*, and *R. mucilaginosa* were characterized by a long lag phase: visible growth on the surface of the alkaline solid medium appeared after 1–2 weeks of incubation (table).

All the alkalitolerant isolates that were originally isolated on alkaline solid medium were also capable of growing on acidic wort agar.

Thus, the alkalitolerant isolates of various yeast species that we obtained from alkaline soil samples turned out to be capable of growing within a wide pH range, suggesting that no yeast fungi are characterized by obligate alkaliphily.

DISCUSSION

Soda-rich saline soils (solonchaks) are habitats characterized by unfavorable environmental conditions. A high pH value, a high degree of mineralization of the soil solution, a wide range of temperature fluctuations, soil freezing during the winter season, and soil thawing and dessiccation during the spring and summer seasons provide for the peculiar composition of the microbial community. Representatives of the halophilic

archaea *Natronobacterium* and *Natrononococcus* [4, 6], spore-forming bacteria of the *Bacillus firmus–B. lentus* group, and the alkaliphilic bacillus *B. horti* [4] were revealed in these habitats. The obligate alkaliphile *Heliospira daurica* [12] also belongs to the inhabitants of the soils. In general, however, the biodiversity of soda-rich soils has not yet been investigated in sufficient detail.

The species diversity of the yeast populations of the soda-rich saline soils studied seems to be relatively insignificant, and it only includes alkalitolerant representatives of asporogenic capsulated yeasts belonging to the genera *Cryptococcus* and *Rhodotorula*. No ascomycetous yeast species were found in samples of these alkaline soils.

It was earlier shown by Chernov *et al.* [13, 14] that the yeast diversity of Arctic tundras and subtropical deserts is more limited than that of forest ecosystems. Yeasts of the genera *Cryptococcus* and *Rhodotorula* account for a very significant percentage of the yeast populations in unfavorable climatic regions. Ascomycetous yeasts are scarce in these regions. This situation exemplifies the Tinemann rule, which predicts that the diversity of living organisms becomes more limited under extreme conditions.

The alkalitolerant yeasts isolated by us were clearly dominated by representatives of the species *C. laurentii*. They were the most abundant kind of yeast, and their numbers exceeded those of other species by two to three orders of magnitude. Hence, *C. laurentii* prevailed in the yeast populations of the soda-rich saline soils studied. Other yeast species only occurred as minor components of the yeast community. The presence of such species as *R. glutinis*, *R. mucilaginosa*, and *S. roseus* is presumably due to their migration into the soil from the surface of halophytes that constitute the plant stratum of the soda-rich solonchak ecosystem.

The species *C. laurentii* is widespread in nature, and its representatives can be isolated from a wide variety of habitats, including palm and muscatel wines, the leaves of tropical plans, seawater, air, frozen beans, human bronchi, and soil [11]; i.e., *C. laurentii* is an eurytopic species. The species is known to be heterogeneous phenotypically and genotypically. The G + C molar ratio varies among the strains of this species within the wide range of 51.2 to 59.7 mol % [11]. It is widely accepted that most eurytopic species are heterogeneous in phenetic and phylogenetic terms and can be subdivided into a number of species occurring in more limited areas [15].

The point that remains to be clarified is whether the alkalitolerant isolates of *C. laurentii* are typical representatives of this species or represent a separate ecotype.

In general, the yeast populations of the soda-rich saline soils studied are characterized by a monodominant pattern (*C. laurentii* clearly prevails over the rest of the species involved) and a low biodiversity level, which is usually characteristic of yeast communities formed under extreme conditions.

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