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EXPERIMENTAL ARTICLES

The Ability of Saprotrophic Bacteria Isolated from Natural Habitats to Lyse Yeasts

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Abstract—More than 600 bacterial strains isolated from different horizons of steppe biogeocenoses and zoogenous loci (diplopod intestines and feces) were tested for the ability to lyse yeast cell walls. About half of the strains that were isolated from biotopes with active degradation of plant debris (steppe litters and diplopod intestines and feces) were found to possess yeast-lytic activity. Most of the yeast-lytic strains belonged to the genera *Streptomyces, Promicromonospora, Oerskovia*, and *Agromyces*. The yeast-lytic activity of actinobacteria from the genera *Agromyces, Mycobacterium*, and *Micrococcus* has not previously been reported.

Key words: yeast-lytic bacteria, soils, plants, zoogenous substrates.

One of the major factors regulating the composition and functioning of microbial communities in natural ecosystems is the interaction of particular species. Such interactions can be either beneficial or antagonistic (an example of the latter interactions is the lysis of some microorganisms by others). The ability of bacteria and actinomycetes to lyse bacterial cells, yeasts, and fungal mycelium is due to their production of extracellular glucosidases, chitinases, lipases, and other lytic enzymes [1–3].

The study of the ecological and geographical distribution of various soil microorganisms made it possible to reveal the biotopes and horizons with the maximum diversity of lytic bacteria (forest and steppe litters) and lytic yeasts (green and dead plant debris) [4–6]. The intestines and feces of soil invertebrates turned out to be favorable for the development of not only bacteria but also yeasts [7, 8].

The aim of this work was to study the ability of bacterial strains isolated from plant and zoogenous substrates to lyse yeasts.

MATERIALS AND METHODS

The tested bacterial strains (a total of 628, belonging to 19 genera) were isolated from different horizons of steppe biogeocenoses and zoogenous loci (diplopod intestines and feces) (Table 1). The yeast-lytic activity of these strains was studied by using five to seven different strains of each of the nine yeast species listed in Table 2. The species *Debaryomyces vanriji, Lipomyces kononenkoae, Lipomyces tetrasporus, Phaffia rhodozyma, Cryptococcus albidus, Rhodotorula glutinis*, and *Sporobolomyces roseus* were isolated from natural biotopes. Two yeast species (*Candida maltosa* and *Saccharomyces cerevisiae*) were obtained from the collection at the Department of Soil Biology.

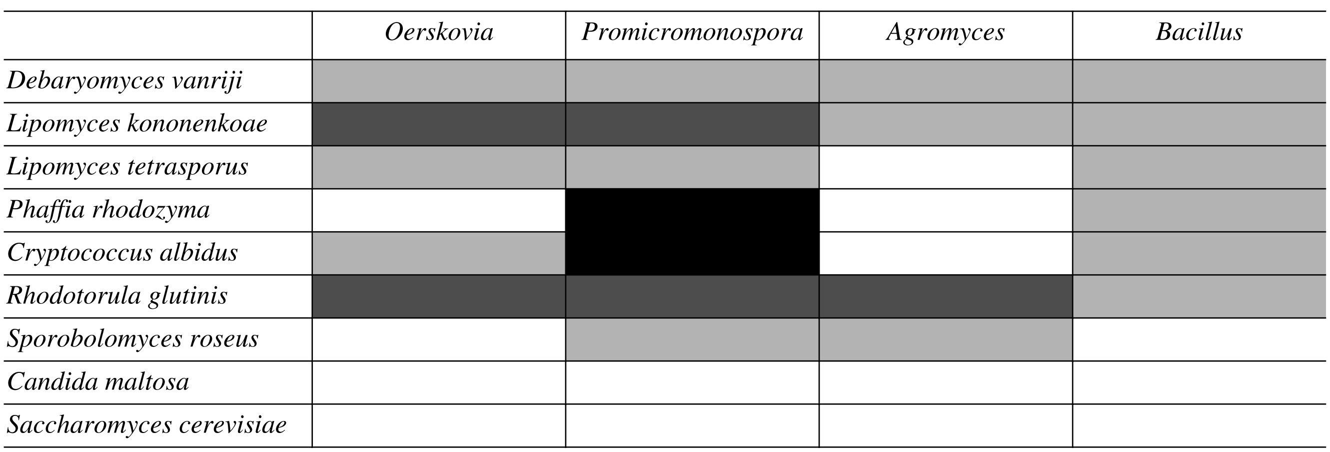
The yeast-lytic activity of bacteria was assayed by cultivating them on agar media with test yeast cells as the sole source of carbon. The test yeast cells were grown on a shaker in flasks containing 200 ml of a medium of the following composition (%): glucose, 3; $(NH_4)_2SO_4$, 5; KH_2PO, 0.085; K_2HPO_4, 0.015; $Mg_2SO_4 \cdot 7H_2O$, 0.05; NaCl, 0.01; $CaCl_2$, 0.01; and yeast autolysate, 0.5. Mesophilic and psychrophilic yeast strains were cultivated, respectively, at 26–28 and 10°C for 2–3 and 10–14 days (to the stationary growth phase). Yeast cells were harvested by centrifugation, washed twice with sterile distilled water, and suspended in molten salt agar (40–60°C) containing (%) $(NH_4)_2SO_4$, 0.1; KH_2PO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 0.05; and agar, 2 (pH 7.0). Five hundred milliliters of the agar medium contained yeast cells from 12 cultivation flasks, which provided a concentration of yeast cells of about 0.7–1%. In some experiments, the agar medium contained the same amount of yeast cells killed by heating at 80°C for 10 min. The agar plates containing yeast cells were inoculated by plating 3-day-old bacterial cultures and incubated at 24°C for 14 days, after which the translucent zones of lysis of yeast cells around bacterial colonies were evaluated. The yeast-lytic activity of bacterial strains was defined as the diameter of such zones.

Table 1. The distribution of yeast-lytic bacteria among various genera

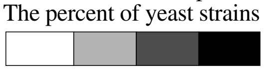
Source of bacteria	Bacterial genera	Number of strains belonging to particular genera	Percent of yeast-lytic strains
Phylloplane and the parietal intestinal community of diplopods	*Micrococcus*	30	27
	Rhodococcus	36	0
	Curtobacterium	4	0
	Pseudomonas	20	0
	Erwinia	15	0
	Serratia	5	0
	Plesiomonas	20	0
	Vibrio	20	0
Plant debris, steppe litters, and the intestinal contents and feces of diplopods	*Promicromonospora*	64	81
	Streptomyces	40	85
	Oerskovia	111	68
	Mycobacterium	83	42
	Cytophaga	20	25
	Myxococcus	30	17
	Cellulomonas	30	0
Soil	*Bacillus*	56	54
	Agromyces	12	75
	Arthrobacter	30	0

Note: The percent of yeast-lytic bacteria was calculated without allowing for the number of susceptible yeast species and the state of yeast cells (either live or dead).

Table 2. The susceptibility of various yeast species to bacteria of four genera



Note: Five strains of each species (a total of 45 strains) were tested for susceptibility to lysis by the particular bacterial strains listed in Table 1. The percent of yeast strains susceptible to bacterial lysis is indicated by shades from white to black according to the following scale:



0–20 20–50 50–70 70–100

RESULTS AND DISCUSSION

Among the bacterial strains that were isolated from the green parts of plants and diplopod gut walls, the fraction of yeast-lytic bacteria was low (approximately 5%). At the same time, about 18% of the bacterial strains isolated from soil were able to lyse yeasts. The greatest percent (about 50%) of yeast-lytic bacteria was revealed among the strains that were isolated from steppe litters and diplopod intestinal contents and feces. Most of the yeast-lytic bacteria belonged to the genera *Streptomyces, Promicromonospora, Oerskovia*, and *Agromyces* (Table 1).

The maximum lytic zones (8–10 mm in diameter) were observed for representatives of the genera *Streptomyces* and *Agromyces.* The yeast-lytic activity of actinobacteria from the genera *Agromyces, Mycobacterium*, and *Micrococcus* has not previously been described (it should, however, be noted that the diameter of lytic zones for these genera did not exceed 1 mm). None of the five other actinomycete genera showed yeast-lytic activity. Among gram-negative bacteria, only cytophages and myxobacteria possessed such activity. The other proteobacteria studied (both aerobic and facultatively anaerobic) did not show yeast-lytic activity (Table 1).

Most of the active bacterial strains could lyse both ascomycetous and basidiomycetous yeasts. The basidiomycetous yeasts *Rhodotorula glutinis, Cryptococcus albidus*, and *Phaffia rhodozyma* and the ascomycetous soil yeasts *Lipomyces kononenkoae* and *L. tetrasporus* were found to be the most susceptible to lysis (Table 2).

In some cases, the ability of bacteria to lyse yeasts was genus-, species-, and even strain-specific. For instance, bacteria of the genus *Agromyces* were able to lyse most strains of the yeast species *Rh. glutinis* and *Sporobolomyces roseus* but were unable to lyse *Cr. albidus* and *Ph. rhodozyma* (Table 2). Bacteria of the genus *Promicromonospora* were able to lyse all strains of the yeast species *Cr. albidus* and *Ph. rhodozyma.* All tested strains (23) of the genus *Agromyces* were able to lyse five of the seven tested strains of *Sp. roseus*, but the other two yeast strains turned out to be resistant to lysis.

In general, the yeasts isolated from natural sources were more susceptible to bacterial lysis than the industrial yeasts *C. maltosa* and *S. cerevisiae*, which could be lysed by only 20% of the bacterial strains tested.

Most of the bacterial strains lysed dead yeast cells more actively than live yeast cells, although actinobacteria of the genera *Streptomyces* and *Promicromonospora* lysed live yeast cells approximately two times more actively than dead yeast cells.

Thus, the study of more than 600 bacterial strains belonging to 19 genera and isolated from various natural biotopes showed that the representatives of about half (exactly, nine) of these genera were able to lyse yeasts, indicating that this ability is widespread among the bacteria of this collection. Yeast-lytic bacteria, most of which were gram-positive, were dominant in the litters and soddy soil horizons of the biogeocenoses. The yeast-lytic ability of actinobacteria from the genera *Mycobacterium* and *Micrococcus*, which inhabit the green and dead parts of plants [6], has not previously been described. The yeast-lytic bacteria of the genera *Streptomyces*, *Promicromonospora, Oerskovia, Bacillus*, and *Micrococcus* were mainly isolated from the intestinal contents and feces of diplopods, which feed on plant debris. This suggests that the epiphytic yeasts ingested by the diplopods together with plant debris [7, 8] are lysed by the yeast-lytic bacteria of the aforementioned genera.

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