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Mutual Relatıonshıps Between Bacterıa and Fungı of Veterınary Sıgnıfıcance

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ABSTRACT

The relationships of fungi and bacteria can take the form of partnership (mutualism) or, conversely, antagonism. Our aim was to study interspecific interaction between microorganisms isolated from animals including fungi *Candida albicans, C. famata, C. krusei, C. lipolytica, C. parapsilosis, C. guilliermondii, Rhodotorula mucilaginosa, Aureobasidium pullulans, Trichosporon asahii, Malassezia pachydermatis* and bacteria *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, S. pseudintermedius, Enterococcus faecalis,* and *Pseudomonas aeruginosa*. The presence of antagonism and mixed-species biofilm formation were studied in 60 combinations of fungi and bacteria. Antagonistic activity was found in only 5 out of 60 fungal-bacterial combinations. *P. aeruginosa* was the most active and showed antagonism towards fungi *A. pullulans, T. asahii,* and *M. pachydermatis*. Biofilm formation in monocultures of yeast fungi was most active in *C. parapsilosis, C. lipolytica* and *T. asahii*. When *C. albicans* was co-cultivated with bacteria, *E. coli* and *S. aureus* strongly stimulated biofilm formation. Out of 60 pairs, mutual fungal-bacterial enhancement of biofilm formation was observed in 9 cases and was most pronounced in combinations of *E. coli* with *C. krusei, C. famata* and *C. lipolytica*; *S. aureus* with *C. famata* and *R. mucilaginosa*; and *E. faecalis* with *C. famata* and *C. lipolytica*. However, in 31 out of 60 combinations, bacteria did not stimulate but inhibited biofilm formation by fungi. *S. pseudintermedius* and *S. aureus* showed the highest inhibitory activity.

Keywords: Fungal-bacterial interaction, Fungal-bacterial biofilms, Antagonism, Biofilms, Veterinary significant microorganisms

INTRODUCTION

Fungi and bacteria are found living together in a wide variety of environments. Their interactions are significant drivers of many ecosystem functions and are important for the health of plants, animals, and humans. Many fungal and bacterial families engage in complex interactions that lead to critical behavioral shifts in the microorganisms. The importance of bacterial–fungal interactions (BFI) in environmental science, medicine, and biotechnology has led to a dynamic and multidisciplinary research field (Deveau et al. 2018).

The interaction of fungi and bacteria during the infectious process is still studied insufficiently in human and veterinary medicine. Fungal and bacterial infections are usually considered separate nosological forms, although in many cases, a combined fungal-bacterial infection (co-infection) occurs (Zhao et al. 2021). This circumstance affects both the pathogenesis of the disease and the approaches to its treatment.

The association of fungi and bacteria may take the form of partnership (mutualism), promoting successful colonization of the host tissues and the development of co-infection. However, at the same time, fungi and bacteria can antagonize and compete for a certain biotope in the body (Kobayashi et al. 2009). However, interspecific relationships between clinically significant bacteria and fungi have only recently begun to be actively studied.

Bacteria and fungi can indirectly affect each other by sensing and responding to diffusible signals such as chemo-attractants, quorum-sensing molecules, and volatile substances. However, several BFIs require a close vicinity and even direct contact between the interacting partners. Various examples of phenotypic adaptation during BFI have been described in the literature, including chemotaxis towards the fungal hyphae, induction of secondary metabolites, attachment, and biofilm matrix production by a bacterium on the hyphae, and facilitation of bacterial movement along the mycelia (Richter et al. 2024).

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More often, fungal pathogens in the affected tissues are present not isolated but in association with one or more bacterial species. For example, it was found that 27% of hospital-acquired bloodstream infections caused by *Candida albicans* were polymicrobial and included gram-positive and gram-negative bacteria (Klotz et al. 2007). Such associations of microorganisms can form complex communities - polymicrobial biofilms, which play a significant role in the pathogenesis of infectious diseases. Infections caused by such biofilms usually progress more severely and are more resistant to therapy. Synergistic interaction between two or more microorganism species leads to an effect unachievable for each of these species individually (Hernandez-Cuellar et al. 2022).

The analysis using a scanning electron microscope showed that when forming a polymicrobial biofilm, bacteria S. aureus adheres to the *C. albicans* yeast cells, which in turn attach to the surface (Hernandez-Cuellar et al. 2022). A number of studies have shown that bacteria can enhance biofilm formation and the pathogenicity of *C. albicans* (Costa-Orlandi et al. 2017). In *C. albicans* and *P. aeruginosa*, antagonistic and synergistic interactions can seemingly take place simultaneously. For example, *P. aeruginosa* induces upregulation of *C. albicans* stress pathways, killing hyphal cells. On the other hand, *P. aeruginosa* quorum sensing also promotes fluconazole resistance in *C. albicans* through upregulation of efflux pumps and ergosterol biosynthesis (Short et al. 2023).

In recent years, due to the enhancement of diagnostic methods, the awareness of polymicrobial etiology in infectious diseases has increased. The interaction of fungi and bacteria has been established in various diseases and locations, including respiratory system infections, invasive diseases, skin and mucosal infections, and bloodstream infections (Costa-Orlandi et al. 2017). In cystic fibrosis lungs, polymicrobial infections often occur with the involvement of bacteria such as *Pseudomonas aeruginosa, Staphylococcus aureus, Burkholderia cepacia, Acinetobacter baumannii, Haemophilus influenzae* coexisting with fungi *C. albicans, Aspergillus fumigatus*, and Scedosporium species (Dixon et al. 2015).

Fungi play an active role within polymicrobial biofilms in infected chronic wounds, although their influence in chronicity and clinical management is not fully understood. The presence of *C. albicans* within an interkingdom chronic wound biofilm was identified as a driving force behind antimicrobial tolerance, highlighting the importance of fungi in wound biofilms and why targeting the fungal scaffold within these biofilms may yield more positive treatment outcomes (Short et al. 2023). However, additional research is needed to understand how these interactions occur and to determine the role of biofilm communities in these infections.

It is important to acknowledge that biofilms are involved in a number of infections in animals. Since the 1980s, biofilm-related infections in domestic animals have been researched, with accentuation on the infections with the largest economic burdens (e.g., mastitis, endometritis, and respiratory infections). The biofilms have been observed in the auditory, cardiovascular, central nervous, digestive, integumentary, reproductive, respiratory, urinary, and visual system. The long-term protection of microorganisms in biofilms can contribute to chronic subclinical infections (Nesse et al. 2023).

The importance of polymicrobial infections is now recognized as a serious problem, but the study of the joint fungi-bacteria biofilm formation is still underappreciated. Knowledge of the role of biofilms in the pathogenesis of animal infections should be expanded by more studies specifically designed to address this question. Among fungi, biofilm formation by *C. albicans* is most studied, while other species of the genus Candida, as well as other clinically significant fungi (Rhodotorula, Aureobasidium, Trichosporon, etc.) are much less studied in this aspect.

The aim of the study was to investigate interspecific relationships between bacteria and fungi isolated from clinically affected animals. The objective of the study included the detection of antagonism (mutualism) between fungi of the genera Candida, Rhodotorula, Aureobasidium, Trichosporon and prevalent bacteria, as well as their mutual influence on the formation of polymicrobial biofilms.

MATERIALS AND METHODS

The research was carried out at the Laboratory of Mycology and Antibiotics n.a. A.Kh. Sarkisov, All-Russian Scientific Research Institute of Experimental Veterinary Medicine n.a. K.I. Skryabin and Y.R. Kovalenko (Moscow, Russia) in 2023–2024. The study used bacterial and fungal strains from the laboratory collection, obtained by the authors during veterinary diagnostic studies. The following strains of bacteria of veterinary origin were used: 1. *Escherichia coli* M144-22, 2. *Klebsiella pneumoniae* M318-22, 3. *Staphylococcus aureus* M203-22, 4. *Enterococcus faecalis* M6-23, 5. *P. aeruginosa* M1-23, and 6. *Staphylococcus pseudintermedius* M7-23.

The yeast fungi were represented by the following strains: 1. *C. albicans* M40/2-22, 2. *Candida famata* M449, 3. *C. famata* M43, 4. *Candida krusei* 431, 5. *Candida lipolytica* 2455, 6. *Candida parapsilosis* 2878, 7. *Candida guilliermondii* M456-22, 8. *Rhodotorula mucilaginosa* M608-22, 9. *Aureobasidium pullulans* M711-22, 10. *Trichosporon asahii* M485-22, and 11. *Malassezia pachydermatis* M204-22. The species identification of microorganisms was performed by MALDI-TOF analysis.

The strains were cryogenically stored in the stock collection at -70°C. After thawing and reactivation, the fungal strains were maintained by subculturing on Sabouraud agar (HiMedia Laboratories, India), and the bacteria on Columbia agar with 5% sheep blood (HiMedia Laboratories, India).

To study the antagonism between fungi and bacteria, the Kirby-Bauer method (the disk-diffusion method) as modified by Lee (2020) was used. Planktonic cultures of bacteria were grown on LB (Luria-Bertani) medium at 37°C for 24h, after that a bacterial suspension with an optical density of 0.5 McFarland was prepared using a Den-1B densitometer (BioSan, Latvia).

Petri dishes with Sabouraud solid medium (HiMedia Laboratories, India) were inoculated with a 0.5mL

suspension of fungi (OD=0.5 McFarland), distributing the suspension evenly over the entire surface with a spatula. The inoculum was allowed to absorb for 10-15min.

Sterile paper disks (HiMedia Laboratories, India) were immersed in a bacterial suspension for 5 seconds, after which the disk was removed and placed on the surface of the Sabouraud agar inoculated with a yeast fungal culture. Five to six disks impregnated with different bacterial strains were placed on one dish. Disks impregnated with saline solution were used as negative control. Disks impregnated with 10% sodium hypochlorite solution were used as positive control.

Incubation of the inoculated plates was carried out at 37°C for 24-48h, until a uniform fungal lawn was formed. The presence of antagonism was assessed by the inhibition of fungal growth around the impregnated disks. If inhibition was observed, the width of the growth inhibition zone was measured in millimeters.

Additional screening for evaluating the antagonism between fungal and bacterial cultures was performed by perpendicular streak method of Madigan et al. (1997). Isolates were screened for antagonism by inoculating a single streak of the fungus in the middle of the Columbia agar with 5% sheep blood plate. Then the plates were subsequently inoculated with bacterial strains by a single streak at a 90 angle to the streak of the fungal strain and finally the plates were incubated for 2-3 days at 37°C. The microbial interactions were analyzed by determining the distance of inhibition measured in mm.

To study biofilm formation, bacteria and fungi were grown in brain heart infusion broth (BHIB) (Sifin, Germany) at 37°C for 24h. Then, 100μL was transferred to 10mL of fresh sterile BHIB. After 6-8h of incubation at 37°C, 10μL of the suspension was transferred to a well with 190μL of BHIB (when studying biofilm formation of monocultures). The 96-well microplates (Sarsted AG, Germany) were used.

In mixed cultivation (fungi + bacteria), 10μL of each species suspension was taken and added to a well containing 180μL of fresh BHIB. After adding the cultures to the wells, the inoculated plates were cultivated for 48h at 30°C.

At the end of the cultivation, the wells were washed 3 times with distilled water and dried. Then 200μL of 0.3% crystal violet solution was added to each well for 10min, then it was drained and the wells were washed with distilled water. They were dried for 3 hours, then 200μL of 70% ethanol was added to extract the dye. The intensity of staining was determined by optical density on a Statfax 4300 photometer (Awareness Technology) at a wavelength of 630nm. All experiments were repeated three times, and the median was determined.

RESULTS

The results of studies on the antagonism between fungi and bacteria using the impregnated disc method are presented in Table 1.

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Table 1: Antagonism of fungi and bacteria. Zones of fungal inhibition around discs with bacterial cultures (mm).

Fungal strains	Bacterial strains and correspondent zones of fungal inhibition, mm					
	E. coli	*S. aureus*	*K. pneumoniae*	*E. faecalis*	*P. aeruginosa*	*S. psedintermedius*
C. albicans M40/2-22	0	0	0	0	0	0
C. famata M449	0	0	0	0	0	0
C. krusei 431	0	0	0	0	0	0
C. lipolytica 2455	0	0	0	0	0	0
C. parapsilosis 2878	0	0	0	0	0	0
C. guilliermondii M456-22	0	0	0	0	0	0
R. mucilaginosa M608-22	0	0	0	0	0	0
A. pullulans M711-22	4±2	0	0	0	6±2	5±2
T. asahii M485-22	0	0	0	0	2±1	0
M. pachydermatis M204-22	0	0	0	0	12±3	13±3

Table 2: Biofilm formation by mixed cultures of fungi and bacteria (intensity in OD units)

	Fungi (monocultures)	*E. coli*	*S. aureus*	*K. pneumoniae*	*E. faecalis*	*P. aeruginosa*	*S. psedintermedius*
Iteration 1							
Bacteria (monocultures)	-	0.279	0.245	0.224	0.228	0.509	0.219
C. famata M449	0.249	0.280	0.287	0.244	0.270	0.268	0.233
A. pullulans M711-22 Cf	0.232	0.269	0.231	0.229	0.234	0.253	0.225
R. mucilaginosa M608-22 Rh	0.241	0.259	0.258	0.229	0.235	0.219	0.232
Iteration 2							
Bacteria (monocultures)	-	0.236	0.217	0.221	0.223	0.255	0.218
C. guilliermondii M456-22 Fc	0.250	0.279	0.222	0.286	0.231	0.252	0.225
C. krusei 431	0.242	0.281	0.217	0.227	0.232	0.234	0.220
C. famata M43	0.229	0.265	0.215	0.221	0.217	0.243	0.212
Iteration 3							
Bacteria (monocultures)	-	0.322	0.221	0.222	0.223	0.249	0.216
C. lipolytica	0.305	0.419	0.216	0.285	0.350	0.259	0.233
C. parapsilosis	0.334	0.288	0.219	0.230	0.277	0.267	0.242
T. asahii M485-22	0.261	0.268	0.225	0.248	0.261	0.242	0.228

Note: Green color highlights pairs with synergistic effects. Red color indicates antagonistic effects, where bacteria inhibit biofilm formation by fungi.

To study biofilm formation, bacteria and fungi were grown in brain heart infusion broth (BHIB) (Sifin, Germany) at 37°C for 24h. Then, 100μL was transferred to 10mL of fresh sterile BHIB. After 6-8h of incubation at 37°C, 10μL of the suspension was transferred to a well with 190μL of BHIB (when studying biofilm formation of monocultures). The 96-well microplates (Sarsted AG, Germany) were used.

In mixed cultivation (fungi + bacteria), 10μL of each species suspension was taken and added to a well containing 180μL of fresh BHIB. After adding the cultures to the wells, the inoculated plates were cultivated for 48h at 30°C.

At the end of the cultivation, the wells were washed 3 times with distilled water and dried. Then 200μL of 0.3% crystal violet solution was added to each well for 10min, then it was drained, and the wells were washed with distilled water. They were dried for 3 hours, then 200μL of 70% ethanol was added to extract the dye. The intensity of staining was determined by optical density on a Statfax 4300 photometer (Awareness Technology) at a wavelength of 630nm. All experiments were repeated three times, and the median was determined.

Among the studied fungal strains, antagonism with bacteria was found in strains *A. pullulans* M711-22, *T. asahii* M485-22 and *M. pachydermatis* M204-22. Fungus A. pullulans M711-22 demonstrated antagonism with bacteria *E. coli*, *P. aeruginosa*, *S. pseudintermedius*, while *T. asahii* M485-22 exhibited weak antagonism only with *P. aeruginosa*. *M. pachydermatis* M204-22 was antagonistic with *P. aeruginosa* and *S. pseudintermedius* (Fig. 1). Therefore, only 3 bacterial species showed antagonism with the fungi - *P. aeruginosa* (inhibited *A. pullulans* and *T. asahii*), *S. pseudintermedius* (inhibited *A. pullulans* and *M. pachydermatis*), and *E. coli* (inhibited *A. pullulans*).

At the next stage, the study of antagonism was continued using the cross-streak method. The same strains of bacteria and fungi were used as in the previous experiment. Only *P. aeruginosa* showed antagonistic activity against fungi — it inhibited the growth of *A. pullulans*, *T. asahii*, and *C. parapsilosis*.

Fig. 2. shows partial inhibition of the fungus *C. parapsilosis* by the *P. aeruginosa* strain. Therefore, antagonism was observed only in 5 out of 60 studied fungal-bacterial combinations.

To study the formation of mono- and mixed myco-bacterial biofilms, we measured the optical density of the polymicrobial biofilm obtained in vitro in 96-well microplates using crystal violet method. In the first stage, we studied biofilm formation in monocultures of yeast fungi. The results are presented in Fig. 3.

The intensity of biofilm formation in fungi varied from OD 0.223 in *C. albicans* to 0.334 in *C. parapsilosis*. Next, the mixed biofilm formation by *C. albicans* and various bacterial species was studied. As shown in Fig. 4, synergistic effects on biofilm formation were observed upon co-incubation of *C. albicans* with *E. coli* (an increase in OD compared to monocultures by 0.091), *S. aureus* (an increase in OD by 0.089), as well as with *P. aeruginosa* and *S. pseudintermedius* (weak stimulation). Notably, according to data (Díaz-Navarro et al. 2023), biofilm formation significantly decreased during co-cultivation of *E. coli* and *C. albicans*, whereas biomass formation increased.

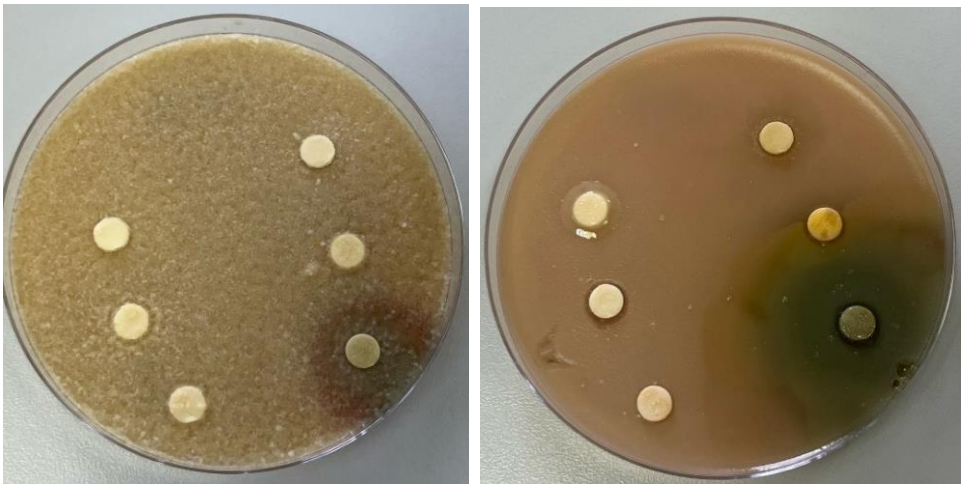

Fig. 1: Growth inhibition zones around the disk with *P. aeruginosa* (bottom right) in *A. pullulans* M711-22 (A) and *T. asahii* M485-22 (B).

Fig. 2: Inhibition of *C. parapsilosis* (vertical streak) by the bacterium *P. aeruginosa*.

The species *K. pneumoniae* and *E. faecalis* did not exert a stimulatory effect on mixed biofilm formation in *C. albicans*. When studying the influence of bacteria of the genus Enterococcus on the ability of *C. albicans* to form biofilms, it was found that *E. faecalis* stimulated the ability to form biofilms, while *E. faecium* reduced the ability to form them (Pashkova 2018).

Next, the influence of bacteria on biofilm formation by fungi was studied in species *C. famata*, *C. guilliermondii*, *C. krusei*, *C. lipolytica*, *C. parapsilosis*, *A. pullulans*, *R. mucilaginosa*, and *T. asahii*. The results are presented in Table 2.

Among the studied bacterial species, *E. coli* most frequently stimulated biofilm formation in fungi, particularly in *C. guilliermondii* (an increase in OD compared to monocultures by 0.029), *C. krusei* (0.039), *C. famata* (0.036), and *C. lipolytica* (0.114).

For S. aureus, besides *C. albicans*, synergistic effects were also observed with *C. famata* (an increase in OD by 0.038) and *R. mucilaginosa* (0.013). These results are consistent with our previous studies on fungal-bacterial associations in clinically affected animals. Most often, Candida spp. formed associations precisely with *E. coli* (24.7%) and staphylococci (25.7%), and much less frequently with other bacterial species (Ovchinnikov et al. 2023).

E. faecalis demonstrated synergy with *C. famata* (an increase in OD by 0.021) and *C. lipolytica* (0.045). *K. pneumoniae* had synergy only with *C. guilliermondii* (0.036). It was revealed that in 31 out of 60 combinations, bacteria did not stimulate but rather suppressed biofilm formation by fungi. Thus, *S. pseudintermedius* suppressed all 9 species of yeast fungi (except *C. albicans*), *S. aureus* - 6 species of fungi, *K. pneumoniae*, *E. faecalis*, *P. aeruginosa* - each 5 species, and *E. coli* - 1 species. In general, stimulating activity was more common among *E. coli*, while inhibitory activity was seen mainly in staphylococci *S. pseudintermedius* and *S. aureus*.

DISCUSSION

The study of fungal-bacterial interaction is a young and relevant research area that has begun to attract the attention of various research groups (Deveau et al. 2018; Zhao et al. 2021; Hernandez-Cuellar et al. 2022; Alshanta et al. 2022; Kahl et al. 2023; Richter et al. 2024).

Modern medicine is currently facing serious challenges in the field of polymicrobial infectious disease treatment, as microorganisms consistently overcome all antimicrobial barriers placed before them. The situation becomes even more alarming considering that microorganisms are often present in the form of biofilms, which are polymicrobial communities. This gives them a competitive advantage, as interactions between different species alter host reactions, reduce the effectiveness of antimicrobial drugs, increase the pathogenicity and virulence of microorganisms, also increasing the severity of infection and promoting resistance to traditional therapy (Seneviratne et al. 2008; Grainha et al. 2020). Mutual fungal-bacterial stimulation of biofilm formation can be one of the mechanisms promoting the formation of a pathological microbiome, leading to the development of the disease and its chronicity (Pashkova 2018).

Microorganisms of veterinary importance are studied in this aspect much less well than microorganisms significant for human infections. In current study, the most common bacteria of veterinary significance were chosen as objects - *E. coli*, *K. pneumoniae*, *S. aureus*, *S. pseudintermedius*, *E. faecalis*, *P. aeruginosa*. As for

fungi,

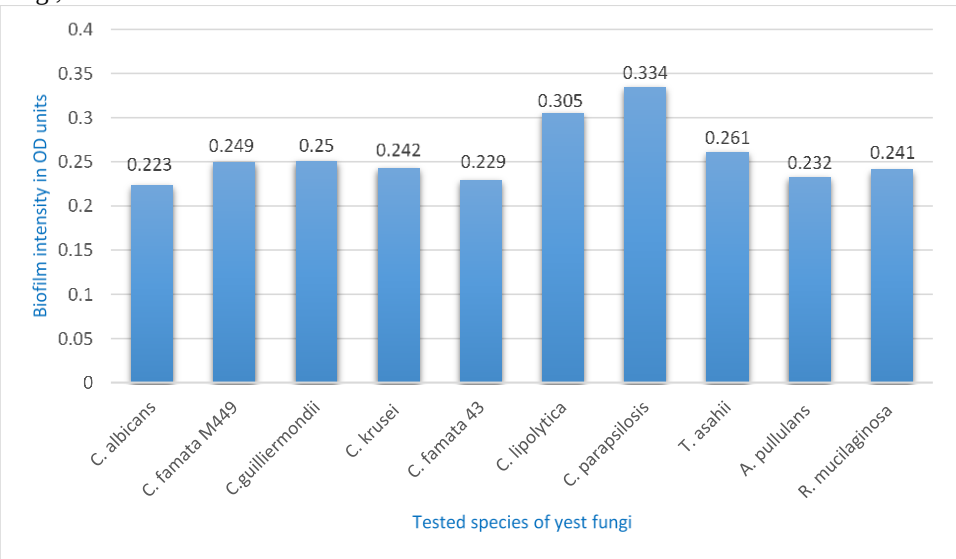

Fig. 3: Biofilm formation by monocultures of yeast fungi. Intensity in OD units.

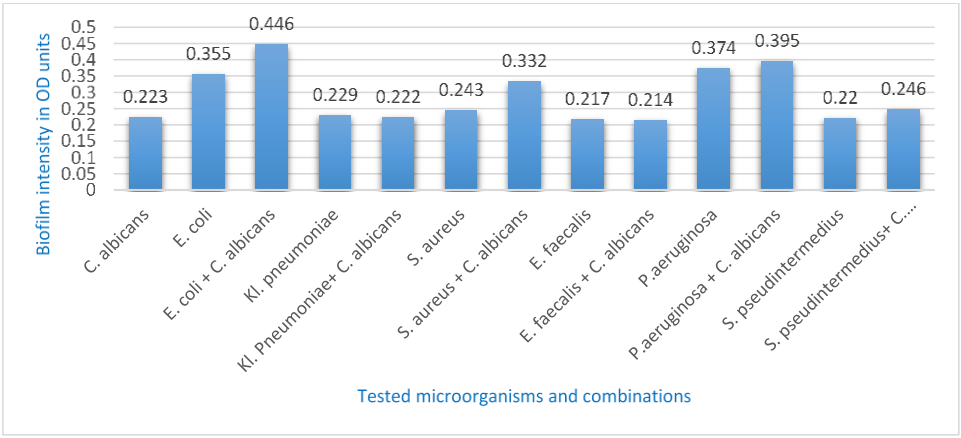

Fig. 4: Biofilm formation by mixed cultures of fungi and bacteria. Intensity in OD units.

besides the well-studied species Candida albicans, other Candida species were studied - *C. famata*, *C. krusei*, *C. lipolytica*, *C. parapsilosis*, and *C. guilliermondii*. Opportunistic fungal pathogens *Malassezia pachydermatis*, *Aureobasidium pullulans*, *Trichosporon asahii*, and *Rhodotorula mucilaginosa* were also included.

When studying antagonism, suppression of fungi by bacteria was found only in 6 out of 60 fungus-bacterial combinations. The species *P. aeruginosa* most frequently exhibited antagonism with fungi. It is noteworthy that *P. aeruginosa* did not show antagonism against *C. albicans*, although some authors have reported antagonism in this pair. Kerr et al. (1994) found that other potentially pathogenic species of Candida (*C. dubliniensis*, *C. glabrata*, *C. guillermondii*, *C. kefyr*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, *C. pseudotropicalis* and *C. tropicalis*) can also be inhibited by Pseudomonas. The fact that clear antagonism was not observed in most pairs of "fungus-bacterium" creates the prerequisites that during the infectious process, the studied prokaryotes and eukaryotes do not suppress each other, but may exhibit synergy.

When studying biofilm formation in monocultures of fungi, the most active biofilm formation was observed in *C. parapsilosis* and *C. lipolytica*. The clinical significance of *C. parapsilosis* has sharply increased in recent years - the species is recognized as the second most frequent in bloodstream infections. Infections caused by *C. parapsilosis* particularly often affect newborns and patients in intensive care units (ICUs). One of the most important factors of virulence of *C. parapsilosis* is biofilm formation, which allows the fungus to colonize medical devices (Pannanusorn et al. 2014).

The clinical significance of *C. lipolytica* is not so evident, however, this species is encountered as a cause of bloodstream infections in individuals with weakened immunity and often causes systemic infections associated with the use of intravenous catheters. In the study by Abbes et al. (2017), it was noted that biofilm formation by *C. lipolytica* was lower (0.25 units) than that of *C. albicans* (0.37 units). However, in our studies, the strain of *C. lipolytica* showed a high level of biofilm formation - 0.30 units. As for other species of Candida (*C. famata*, *C. krusei*, *C. parapsilosis*, and *C. guilliermondii*), they demonstrated biofilm formation at a level of 0.22-0.26 units, which is comparable to that of *C. albicans* (0.22 units).

Fungi *A. pullulans*, *T. asahii*, and *R. mucilaginosa*, along with species of Candida, actively formed biofilms, exceeding *C. albicans* in intensity. Literature data on biofilm formation in these yeast species are quite limited. Di Francesco et al. (2021) report on the ability of A. pullulans to form biofilms. *T. asahii* can also form biofilms, which are 16000 times more resistant to voriconazole than planktonic cells (Di Bonaventura et al. 2006). Biofilm formation has also been detected in *R. mucilaginosa*, which correlates with our results (Gattlen et al. 2011).

When studying biofilm formation by *C. albicans* in association with different species of bacteria, it was found that biofilm formation was significantly enhanced by *E. coli* and S. aureus, while *P. aeruginosa* and *S. pseudintermedius* slightly stimulated biofilm formation. These results are consistent with our previous studies on the associations of fungi and bacteria in clinically affected animals. Species of Candida most often formed associations with *E. coli* (24.7%) and staphylococci (25.7%), and much less frequently with other species of bacteria (Ovchinnikov et al. 2023).

Increased biofilm formation in the *C. albicans*-*S. aureus* pair, which we observed, is also noted by other authors. Postnikova et al. (2018) reports that biofilm formation in a mixture of *C. albicans* and *S. aureus* increases twofold compared to monocultures. Some studies demonstrate that although *S. aureus* forms poor biofilms in monoculture, it actively forms polymicrobial biofilm in the presence of *C. albicans* (Harriott et al. 2009).

The joint biofilms of *C. albicans* and *E. coli* have been studied much worse. In the study of Díaz-Navarro et al. (2023), an increase in biomass and metabolic activity in mixed cultures of *C. albicans* and *E. coli* is shown, which agrees with our data. Other authors report that *E. coli*, on the contrary, inhibits biofilms of *C. albicans* (Samaranayake et al. 2014).

It is worth mentioning that *P. aeruginosa* not only did not inhibit, but on the contrary, stimulated biofilm formation in *C. albicans*. This contradicts studies that revealed antagonism between *C. albicans* and *P. aeruginosa* in vitro (Fourie et al. 2019). Many in vivo studies also demonstrate the simultaneous co-infection of these species in various loci (Kahl et al. 2023).

Bacterial species K. pneumoniae and *E. faecalis* had no significant effect on biofilm formation by *C. albicans*. At the same time, *E. faecalis* and *C. albicans* are often isolated together from various foci of infection. It has been shown that *E. faecalis* inhibits biofilm formation and transition to the mycelial phase in *C. albicans* due to a decrease in pH (Alshanta et al. 2022). According to Pashkova (2018), *E. faecalis* stimulated the ability of *C. albicans* to form biofilms, while E. faecium, on the contrary, reduced it.

Interesting results were obtained when studying the influence of bacteria on Candida non-albicans species, as well as *A. pullulans*, *R. mucilaginosa*, and *T. asahii*. It was revealed that *E. coli* has the widest range of biofilm stimulation in 4 of the 9 studied fungal species, including *C. lipolytica*, *C. krusei*, *C. famata*, and *C. guilliermondii*. According to Bandara et al. (2009), *E. coli* inhibits biofilm formation in *C. krusei*, *C. parapsilosis*, and *C. dubliniensis*, but stimulates it in *C. tropicalis*. Our data coincide regarding *C. parapsilosis* (biofilm inhibition), but do not coincide regarding *C. krusei*, since we observed stimulation of biofilm formation by *E. coli*.

In our study, S. aureus stimulated biofilm formation in *C. albicans*, *C. famata* and *R. mucilaginosa*, and inhibited it in all other Candida non-albicans species. In other studies, it was shown that the culture fluid of Staphylococcus stimulated biofilm formation in *C. albicans* and *Saccharomyces cerevisiae*, but inhibited it in *C. krusei* and *C. parapsilosis*, which coincides with our results. The authors believe that the metabolites of Staphylococcus caused the death of yeast cells through the mechanism of apoptosis, but this mechanism is not activated in *C. albicans* (Camarillo-Márquez et al. 2018).

We were unable to find published data on the stimulating effect of S. aureus on biofilm formation in *C. famata* and *R. mucilaginosa*. On the contrary, some studies that show that the carotenoid pigment of Rhodotorula inhibits *S. aureus* biofilms (Naisi et al. 2023). *S. aureus* inhibited biofilm formation in other Candida non-albicans species - *C. guilliermondii*, *C. lipolytica*, and the fungus T. asahii. The bacterium *K. pneumoniae* stimulated biofilm formation only in *C. guilliermondii* and inhibited it in most other Candida non-albicans species. Comparable data in the literature could not be found.

In sum, for the first time, we have studied biofilm formation in a variety of clinically significant yeasts of veterinary origin and the influence of bacteria on the biofilms of these fungi. The ability to form biofilms in *C. famata*, *C. krusei*, *C. lipolytica*, *C. parapsilosis*, *C. guilliermondii*, *A. pullulans*, *T. asahii*, and *R. mucilaginosa* exceeded that of *C. albicans*. It has been established that associations of fungi and bacteria in certain combinations are capable of mutually enhancing biofilm formation, and therefore such pairs possess greater pathogenic potential during the infection than monocultures of the same species. At the same time, in certain combinations, bacteria exert an inhibitory effect on fungal biofilm formation. Thus, the phenomenon of opposing (enhancing/suppressing) influence of microorganisms is species-specific and should be considered individually for each pair of microsymbionts. The obtained data contribute to understanding the mechanisms of pathogenesis in mixed fungal-bacterial infections, however, further study of intermicrobial interaction and its role in human and animal infectious pathology is necessary. In the future, these findings will be useful for developing therapies and preventive measures for fungal-bacterial infections.

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Authors' contribution

Ovchinnikov R.S. developed the concept of the study, processed and interpreted the results, and prepared a draft of the article. Savinov V.A. and Samylina I.V. conducted a study, collected primary data and edited the article.

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