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ANTI-*STAPHYLOCOCCI* ACTIVITY OF YEAST ISOLATES AFFECTED BY pH OF EXPERIMENTAL MEDIUM

Diverse biological activities of yeasts make them promising candidates for a wide range of applications not limited to the food industry. Traditionally yeasts are involved in the wine, bread, beer, fermented foods manufacturing having a long history of use as a source of protein, organic acids, vitamins and minerals. Yeasts are receiving also increasing interest for the development of biotechnological production of aromatic alcohols, enzyme, xanthines, aromatic amino acids, cholesterol-lowering statins, hypocholesterolemic components, natural pigments and others bioactive compounds [1–5].

In addition to these properties yeasts are known for their antagonistic activities toward undesirable bacteria and fungi. The use of antagonistic bacteria to inhibit pathogenic bacteria has been studied extensively over the years, while little attention has been given to yeasts in a similar role. The study and potential applications of antibacterial compounds produced by yeasts are still at early stage of development. The first positive indications of the activity of yeasts against Gram-positive and Gram-negative bacteria were discovered by Hayduck and Fernbach in 1909. Since then there have been reported antibacterial activity of *Debaryomyces hansenii* against *Clostridium tyrobutyricum* and *Clostridium butyricum*, inhibition of the growth of the beer spoilage bacteria *Bacillus megaterium* and *Lactobacillus plantarum* by *Kloeckera apiculata* and *Kluyveromyces thermotolerans*, inhibition of *Listeria* by a strain of *Geotrichum candidum, Debaryomyces hansenii, Candida intermedia*, *Kluyveromyces marxianus, Pichia norvegensis* [6, 7].

Many yeasts also demonstrated antifungal activity towards other yeast cultures described as the killer phenomenon [8]. However it was no clear evidence that the killer toxins formation directly correlated with antibacterial activity in yeasts [9].

Antagonism of microorganisms by yeasts has been attributed broadly to 1) competition for nutrients; 2) pH changes; 3) production of high concentrations of ethanol; 4) killer toxins or mycocins; 5) mycocin causes ion leakage by the formation of channels on the cytoplasmic membrane; 6) mycocin inhibits the synthesis of cell wall component β-1,3-glucan; 7) mycocin interrupts cell division by blocking the DNA synthesis; 8) proteases degrade bacterial toxins; 9) stimulate the immune response; 10) yeasts inhibit attachment to intestinal cells [7].

The discovery of antagonistic activities of yeasts may have a significant impact in numerous fields such as food, agriculture, medicine, veterinary, environmental protection. Yeasts have been proposed as a new generation of biocontrol agents and basis for new kind of probiotics. Yeasts cultures with pronounced antibacterial properties may serve as an attractive alternative to becoming increasingly controversial antibiotic-based therapy of bacterial infections. It is well known that *Staphylococci* are leading group causing infectious diseases being ubiquitous agents of nosocomial infections. They are evolving resistance to antibiotics or biocides resulting, for example, in the phenomenon of methicillin-resistance. Currently methicillin-resistant *S. aureus* (MRSA) is one of the most greatly feared bacterial strains which resistance is commonly caused by the widespread use of antibiotics in the husbandry of livestock, including prevention or treatment of an infection as well as promoting growth.

The aim of this work was to study the antagonistic activity of yeasts against various isolates of potentially pathogenic bacteria *Staphylococcus aureus* in relation to the acidity of the experimental medium for yeasts growth.

Materials and methods

Yeasts were isolated from the samples of authentic homemade dairy products and from the gastro-intestinal tract (GIT) of healthy Hucul long-livers from Ukrainian Carpathians by using wort agar (1.3% maltose, 1.5% malt extract; 3.0% dextrin, 1.0% K_2HPO_4; 1.0% NH_4Cl; 1.8% agar, pH 7). The bacterial cultures used in this study included standard reference strain *Staphylococcus*

aureus ATCC 25923 from Ukrainian Collection of the Microorganisms and three clinical isolates of *S. aureus*. The organisms were maintained by routine culture on agar slants and stored at 4 °C between transfers. At least two additional subcultures of microorganisms (24 h, 28 °C for yeast and 37 °C for bacteria) were made in fresh medium prior to their use in the experiment.

The pure cultures of yeasts were tested for antagonistic activity towards *S. aureus* strains *in vitro* by using an agar plug method [10]. The agar plugs inoculated on agar plates were initially prepared by plating 1 ml (6 x 10^8 cells mL^{-1}) of yeasts cell suspension on wort agar (20 ml) at different pH values (5.0; 5.5; 6.0; 6.5; 7.0). The plates were incubated at 28ºC for 72 h. Adjustment of the pH was performed using sterile citrate-phosphate buffer before pouring the plates. Agar plugs were prepared by growing the yeasts on wort agar for three days. A sterile 8-mm-diameter cork borer was used to obtain the agar plugs from these culture plates.

The cell suspensions of 24 h bacterial cultures (1 mL, 1.5×10^8 cells mL-1) were pour-plated on meat peptone agar (MPA) (20 ml). Plugs of yeast cultures were placed onto MPA containing a lawn of bacterial strains. After an incubation period of 24 h at 37 °C, the diameter of clear zones surrounding the plugs was measured. The experiment was carried out in triplicates.

Results and discussion

The yeasts screening for their antagonism towards *S. aureus* was performed using fifty-two strains of yeast isolates (table 1).

Of the total yeasts tested, eight cultures were positive for the antagonistic activity comprising 15.4%. Six out eight yeast isolates showed growth inhibition of more than two sensitive *S. aureus* bacterial strains (table 2). The antagonism yeasts towards *S. aureus* was affected by pH of experimental medium for yeasts cultivation showing generally the best performance of this activity at pH values ranging between 6.0 and 7.0.

It is worth mentioning that *Staphylococci* together with *Escherichia coli* were the first microorganisms against which the phenomenon of yeasts antagonism towards bacteria was discovered [7]. Generally the best anti-*Staphylococci* performance in terms of the high values of growth inhibition zone for all tested bacteria at the wide range of tested pH values of experimental medium was observed for *Saccharomyces pastorianus* strains FF7 and FF18 isolated from dairy products. These yeast isolates manifested the highest values of antagonistic activity with diameter of growth inhibition zone reaching 25–28 mm at comparatively low pH values range 5.0–6.0. This may be related to their origin being dairy products and associations with lactic acid bacteria. Microbial evolution of yeasts and lactic acid bacteria populations in dairy products involved the various mechanisms giving microorganisms a competitive advantage. It is well known that synthesis of alcohols is a compensatory mechanism to resist the high acidity stress. In *S. cerevisiae*, for example, citric acid shifted the primary metabolism towards higher glycerol production [11]. In addition to synthesis of alcohols, yeasts produce other metabolites that broadly inhibit cell growth. The yeast adaptations to the conditions of low pH can be used for the selection of probiotic strains for complex probiotics. The behaviour of yeasts in experimental treatments might be similar to those found in commercial products.

The rest of the tested yeast isolates did not manifest any inhibition of *Staphylococci* growth at pH 5.0–5.5. Apart of *Rhodotorula spp.* S1 inhibiting all tested *Staphylococci* reaching the growth inhibition values of 20–25 mm at pH 6.0, all other cultures showed considerably weaker antagonistic activity towards bacteria. The lowest performance was observed for *Candida magnoliae* S13 which was able to inhibit only growth of standard reference *S. aureus* strain reaching only 12 mm of inhibition zone at pH 6.5–7.0 but did not affect any of clinical isolates of *S. aureus*.

Table 1

Yeast isolates and their origin

Yeast strain code	Source and year
F2, F15, F21, F23, F25, F26, F27, F28, F29, F31, F35, F36	Dairy products, 2013
FF1, FF2, FF4, FF5, FF6, FF7, FF8, FF9, FF10, FF11, FF12, FF13, FF14, FF15, FF16, FF18	Dairy products, 2014
S1, S2, 3S, S4, S5, S7, S10, S11, S12, S13, S14, S15, S16, S17, S19, S21, S22, S23, S24, S25, S28, S30, S31, S32	Long-livers GIT, 2013

Table 2

Antagonistic activity of the yeast isolates against *S. aureus* depending on pH values of the experimental medium

Yeast isolate	pH	Diameter of growth inhibition zone (mm)			
		S. aureus ATCC 25923	*S. aureus* 19	*S. aureus* 33	*S. aureus* 38
S1 *Rhodotorula spp.*	5.0	0	0	0	0
	5.5	0	0	0	0
	6.0	24.3 ± 0.5*	22.1 ± 1.7	20.5 ± 2.5	25.1 ± 0.3
	6.5	19.4 ± 0.2	19.1 ± 1.1	17.6 ± 0.3	19.1 ± 0.1
	7.0	16.7 ± 1.6	14.1 ± 0.9	11.2 ± 0.2	14.4 ± 0.7
S2 *Debaryomyces hansenii*	5.0	0	0	0	0
	5.5	0	0	0	0
	6.0	20.5 ± 0.5	0	19.4 ± 0.2	17.1 ± 1.1
	6.5	18.2 ± 0.4	0	15.3 ± 0.7	15.2 ± 1.2
	7.0	13.6 ± 1.3	0	13.3 ± 0.8	12.3 ± 0.9
S3 *Debaryomyces hansenii*	5.0	0	0	0	0
	5.5	0	0	0	0
	6.0	15.5 ± 0.5	13.1 ± 0.1	12.2 ± 1.4	19.5 ± 0.5
	6.5	13.2 ± 0.4	11.3 ± 0.6	11.3 ± 0.3	17.2 ± 0.6
	7.0	12.2 ± 1.2	10.3 ± 0.5	10.8 ± 1.4	15.5 ± 0.5
S5 *Debaryomyces hansenii*	5.0	0	0	0	0
	5.5	0	0	0	0
	6.0	17.2 ± 0.6	0	0	19.2 ± 0.2
	6.5	17.2 ± 0.4	0	0	18.5 ± 0.5
	7.0	15.8 ± 0.4	0	0	18.2 ± 1.2
S11 *Candida spp.*	5.0	0	0	0	0
	5.5	0	0	0	0
	6.0	15.2 ± 0.4	14.2 ± 0.1	14.3 ± 0.6	20.2 ± 0.4
	6.5	14.3 ± 0.8	12.2 ± 0.6	13.2 ± 1.4	15.7 ± 0.4
	7.0	10.1 ± 0.4	11.2 ± 0.2	11.2 ± 0.8	12.2 ± 0.2
S13 *Candida magnoliae*	5.0	0	0	0	0
	5.5	0	0	0	0
	6.0	0	0	0	0
	6.5	12.6 ± 0.4	0	0	0
	7.0	12.2 ± 0.2	0	0	0
FF7 *Saccharomyces pastorianus*	5.0	13.1 ± 0.3	14.2 ± 0.1	10.2 ± 0.2	25.3 ± 0.8
	5.5	24.2 ± 0.4	19.2 ± 0.6	15.2 ± 0.2	25.2 ± 0.1
	6.0	20.2 ± 0.8	19.1 ± 0.4	15.6 ± 0.4	23.5 ± 0.5
	6.5	19.1 ± 0.1	15.2 ± 0.2	15.2 ± 0.4	20.6 ± 1.8
	7.0	16.4 ± 0.4	14.7 ± 0.1	15.1 ± 0.1	20.3 ± 0.3
FF18 *Saccharomyces pastorianus*	5.0	0	0	0	0
	5.5	28.5 ± 0.5	25.2 ± 1.2	23.2 ± 0.4	28.1 ± 0.1
	6.0	28.2 ± 1.1	25.5 ± 0.5	20.4 ± 0.2	26.1 ± 0.2
	6.5	21.3 ± 0.7	19.2 ± 0.6	20.2 ± 0.6	20.5 ± 0.5
	7.0	18.2 ± 0.4	19.1 ± 1.3	17.7 ± 0.1	19.2 ± 0.8

Note: * ± SEM.

Conclusions

In conclusion the anti-*Staphylococci* activity of yeast isolates was species- and strain-depended and affected by pH values of experimental medium for yeast cultivation.

The sources of yeasts isolation in the present study were authentic and safe for the development of the perspective biocontrol agents against *Staphylococci*. The further research on the yeasts dwelling in previously unstudied unique environments is essential not only for potential application of their antagosnism properties towards harmful bacteria and other useful biological properties but also for elucidation of their biodiversity and ecological roles.

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The ***aim*** of this work was to study the antagonistic activity of yeasts isolates towards various strains of potentially pathogenic bacteria *Staphylococcus aureus*. ***Methods.*** Over 50 yeasts isolates from authentic dairy products and gastro-intestinal tract (GIT) of Hucul long-livers from Ukrainian Carpathians were tested for antagonistic activity towards *S. aureus* strains by using a conventional agar plug method at pH values of wort agar ranging from 5.0 to 7.0. ***Results.*** The anti-*Staphylococci* activity was found in 15.4% of tested yeast isolates. In general, the best antagonistic activity towards *S. aureus* was observed for *Saccharomyces pastorianus* isolated from dairy products followed by GIT isolates *Rhodotorula spp.* S1 > *Debaryomyces hansenii* isolates > *Candida spp.* S11 > *Candida magnoliae* S13. The majority of yeasts isolates inhibited tested *S. aureus* strains at pH 6.0–7.0. ***Conlusions.*** Anti-*Staphylococci* performance of yeast isolates was affected by pH values of experimental medium and depended on yeast species and strains.

Keywords: yeasts, *Staphylococcus aureus*, antagonism, pH.