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Bacterial Regulation of Antagonistic Activity of Bacteria

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The phenomenon of microbial regulation of bacterial antagonism was studied using metabolites and cell walls of indicator microorganism culture as inductors. The algorithm of selection of stimulators of bacterial antagonistic activity is determined, experimental conditions are described, and methodological approach to stimulation of bacterial antagonistic activity is developed, which can be used for stimulation of probiotic antagonistic activity and for improving colonization resistance of the host organism during infection.

Key Words: *bacteria; antagonism; regulation; metabolism; cell wall*

Antagonism in the world of bacteria is a highly prevalent phenomenon: one bacterium species suppresses the development or inhibits the growth of other microorganisms (MO) [2]. Antagonistic relationships between bacteria are one of the mechanisms of microbiocenosis formation and maintenance of colonization resistance in human biotopes [1].

The appearance of bacterial antagonism depends on the environmental conditions. The effects of abiotic and biotic environmental factors on antagonistic activity (AA) of bacteria have been studied [2], the mechanism of antagonism autoinduction [8,11] and effects of heterologous metabolites of MO on bacterial AA have been described [6,7,9, 12]. Unfortunately, despite these data, the possibility of bacterial regulation of bacterial AA and its use for infection control remain unsolved problems.

We studied the effects of *Micrococcus luteus* and *Staphylococcus aureus* exometabolites and cell wall fragments on bacterial AA and developed a method for selection of MO antagonism inductors.

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MATERIALS AND METHODS

Antagonist strains were selected from MO isolated from the vaginal biotope and nasal cavity of normal subjects and bacteria carriers: *S. aureus*, *Enterococcus faecalis*, *Corynebacterium minutissimum*, *Lactobacillus casei*, and clinical strains of *Bacillus subtilis* and *Pseudomonas aeruginosa* from the collection of Institute of Cellular and Intracellular Symbiosis. The bacteria were identified routinely by morphological, tinctorial, cultural, and biochemical characteristics [4] using Api ID 32 Staph and Rapid ID 32 Strep kits (Bio Meriex). *S. aureus* isolated from the anterior nasal cavity and *M. luteus* No. 2665 (L. A. Tarasevich Institute of Standardization and Control) served as indicator cultures.

Lactobacteria were cultured in Moser—Rogoz—Sharp medium (MRS; HiMedia), other MO were grown in meat-peptone broth (MPB; Nutrient Media Company).

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Constitutive antagonism was detected by the method of delayed antagonism in solid nutrient medium after Fredericq [10], inducible antagonism by the dish method of direct antagonism after Murray [13] if the sign was not detected by Fredericq method. The effects of indicator strains on AA of the studied cultures were evaluated by a modified method of delayed antagonism in liquid nutrient medium [5], in which the culture fluid of the antagonist treated by *M. luteus* or *S. aureus* components (exometabolites and cell wall) was tested. Chloroform-treated supernatant (exometabolites) and cell wall of the indicator culture obtained by successive treatment of the indicator strain biomass by ethanol-chloroform mixture, aqueous solution of sodium hydroxide, acid buffer solution of trypsin (after alkali neutralization), ethanol, and acetone, were used. Cell wall was autoclaved at 0.5 atm for 30 min. The indicator strain cell wall suspension opacity was equal to the optical density of the broth culture. Changes in the studied antagonist culture AA were evaluated by changes in the indicator strain survival after exposure to metabolites of the antagonist treated with components of the indicator strains *M. luteus* or *S. aureus* in comparison with metabolism of the antagonist treated with MPB. The effects of chloroform (used as sterilizing material in the delayed antagonism methods) on the antagonist metabolites were ruled out by comparing the activities of supernatants of antagonist strains disinfected with chloroform (experiment) and by filtration (Millipore, 0.22 μ; control).

The results were processed using Student—Fisher test.

RESULTS

Studies by methods of direct and delayed antagonism showed that AA of the studied cultures was revealed mainly by the method of direct antagonism (inducible type; Table 1). Low incidence of detection of activity by the method of delayed antagonism excluding bacterial induction of the sign was not associated with degradation of antibacterial substances under the effect of chloroform, which attested to constitutive type of antagonism. Further studies showed regulatory effect of exometabolites and cell wall of MO in antagonism of bacteria of different taxons.

Antagonism of enterococci, lacto- and corynebacteria, bacilli, *P. aeruginosa*, staphylococci was observed with *M. luteus* indicator strain (Table 1, Fig. 1, *a*).

AA of all studied *E. faecalis* increased after treatment with *M. luteus* components. AA of corynebacteria and bacilli increased after addition of micrococcus cell wall into the culture medium. AA of lactobacteria and *P. aeruginosa* to *M. luteus* was high and did not change after treatment with its components. The studied micrococcus antagonists, *S. aureus* strains, were divided into 2 groups: *S. aureus* 1 and *S. aureus* 2. In the control, *S. aureus* 1 strains (75% strains) stimulated the growth of *M. luteus*. AA manifested after their treatment with micrococcus components and was stronger after treatment with *M. luteus* exometabolites than cell wall. *S. aureus* 2 (25% strains) in the control exhibited AA to the micrococcus, which increased only after their treatment with *M. luteus* exometabolites.

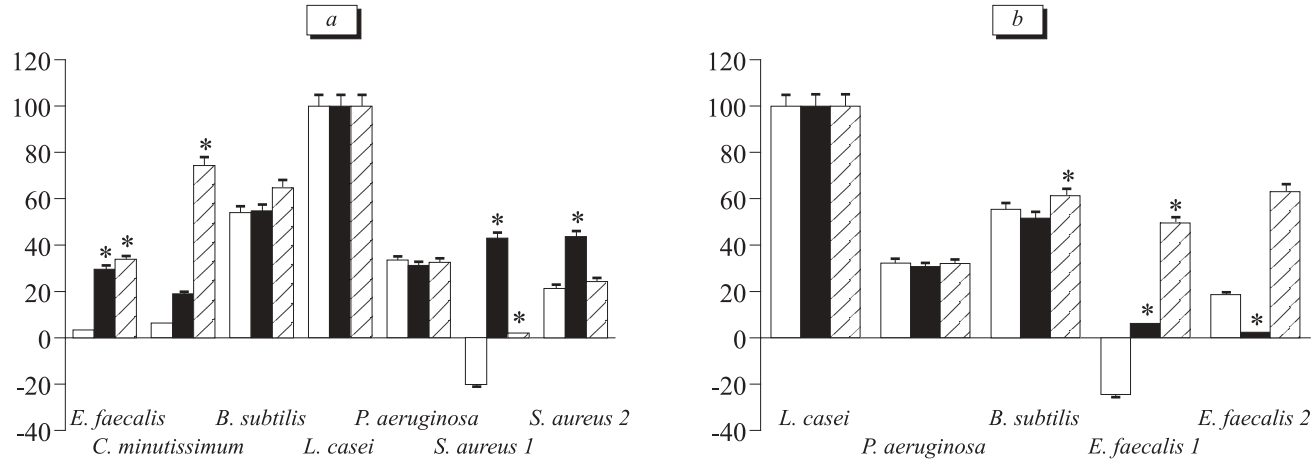

Fig. 1. Intensity of bacterial AA to *M. luteus* (*a*) and *S. aureus* (*b*) depending on the presence of their exometabolite and cell wall in the antagonist culture medium. Ordinates: AA of the studied cultures in % of the indicator strain suppression (values below zero show the indicator culture growth stimulation). Light bars: control; dark bars: effects of antagonist cultures with the indicator strain exometabolites; cross-hatched bars: effects of antagonists cultured with the indicator strain cell wall. *$p<0.05$ compared to the control.

TABLE 1. Incidence of Antagonistic Activity (%) in Bacteria of Different Taxons

Antagonist	Method for antagonism evaluation					
	direct antagonism in solid nutrient medium after Murray [13]		delayed antagonism in solid nutrient medium after Fredericq [12]		delayed antagonism in liquid nutrient medium after Bukharin [4], modified method	
	to *M. luteus*	to *S. aureus*	to *M. luteus*	to *S. aureus*	to *M. luteus*	to *S. aureus*
S. aureus (*n*=12)	100	—	25	—	100	—
C. minitissimum (*n*=3)	100	0	0	0	100	0
E. faecalis (*n*=12)	100	100	0	0	83.3	100
L. casei (*n*=5)	—	100	0	40	100	100
B. subtilis (*n*=3)	100	100	100	100	100	100
P. aeruginosa (*n*=6)	100	100	100	100	100	100

Note. Dash: no AA observed because the conditions of culturing did not fit *M. luteus*.

Antagonism of lactobacteria, *P. aeruginosa*, bacilli, and enterococci was observed with *S. aureus* as the indicator strain (Table 1; Fig. 1, *b*).

AA of *L. casei* and *P. aeruginosa* to *S. aureus* was high and did not change under the effects of its components. AA of *B. subtilis* increased after addition of *S. aureus* cell wall to the antagonist culture medium. The studied *E. faecalis* strains were divided into 2 groups by their effect on *S. aureus*. *E. faecalis* 1 (25% strains) stimulated the growth of *S. aureus* in the control. AA appeared after treatment with *S. aureus* components, increasing after addition of *S. aureus* cell wall to the culture medium. *E. faecalis* 2 (75% strains) exhibited AA to *S. aureus* in the control, which also increased after their treatment with *S. aureus* cell wall. Treatment with exometabolites reduced stimulation of *S. aureus* growth in the presence of *E. faecalis* 1, observed in the control, up to the development of inhibitory effect, while for *E. faecalis* 2 strain, treatment with exometabolites led to reduction of AA.

Stimulation of AA by the indicator culture components was characteristic of *S. aureus* 2, *E. faecalis* 2, and *B. subtilis* strains with constitutive antagonism. *S. aureus* 1, *E. faecalis* 1, and *C. minutissimum* strains with inducible antagonism exhibited manifest activity [6] in response to addition of the indicator bacteria components to the antagonist culture medium. The modification of the method [5] identified different types of antagonism by the type of expression and rendered some advantages to the original method: more frequent detection of AA in comparison with the method of delayed antagonism and differentiation of antagonism regulators in comparison with the method of direct antagonism.

Inducible antagonism is prevalent in MO symbiont bacteria [6,7-9,11], which is presumably a characteristic sign of indigenous flora. It is also possible that stable trophic relationships develop in evolutionally fixed and formed communities; competitive relationships initiating antagonism in indigenous MO appear during biotope colonization by allochtonic flora [12]. Constitutive antagonism is intrinsic of free species, such as *Bacillus sp.* and *Pseudomonas sp.*, usually habituating under conditions of the substrate insufficiency and hence, stringent competition. Manifestation of the sign was constant for lactobacteria, realizing their antagonism at the expense of acid and H_2O_2 and at the expense of antibiotics, whose synthesis can be induced in lactobacilli [8].

Hence, MO can regulate manifestation of AA by bacteria of different taxons, which can be one of the mechanisms of microbiocenosis formation and stable colonization resistance. Presumably, due to this phenomenon we can regard antagonism not just as the capacity of bacteria to suppress the development or inhibit the growth of other MO, but also as a manifestation of associative symbiosis [1]. The indicator culture exometabolites and cell walls exhibited a regulatory effect towards the studied cultures AA; they can be used for stimulation of the probiotic antagonistic activity and increase of the host colonization resistance. The method for evaluation of bacterial antagonism, described in this paper, can be used for selection of AA inductors for indigenous microflora representatives.

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