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EXPERIMENTAL ARTICLES

The Effect of the Intra- and Extracellular Metabolites of Microorganisms Isolated from Various Ecotopes on the Catalase Activity of *Staphylococcus aureus* ATCC 6538 P

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Abstract—The cell extracts (i.e., intracellular metabolites) and culture liquids (i.e., extracellular metabolites) of microorganisms isolated from various ecotopes were found to inhibit the catalase activity of *Staphylococcus aureus* ATCC 6538 P, which resulted in a considerable inhibition of the growth of metabolite-treated *S. aureus* cells by hydrogen peroxide. The inhibitory effect of microbial metabolites on *S. aureus* catalase can be considered as a mechanism of intercellular interactions responsible for the formation of microbiocenoses.

Key words: *Staphylococcus aureus*, catalase, microbial inhibitors.

Interbacterial interactions, antagonistic in particular, are essential in the formation of microbial biocenoses [1, 2]. Microbial antagonism is due to the production of lysozyme [3], organic acids [4], bacteriocins [5], and hydrogen peroxide [6]. The regulatory effect of hydrogen peroxide on the intracellular level of low-molecular-weight thiols and potassium ions mediated through the inhibition of membrane transport and the protective role of catalase in bacterial defense against oxidative stress have been described in a number of publications [7–10]. It remains, however, unclear whether or not exogenous microbial metabolites can affect the antioxidant protective systems of bacteria, including catalase. The answer to this question would provide insight into the mechanisms of intercellular interactions and their role in the formation and maintenance of microbial biocenoses.

The aim of the present work was to study the effect of exogenous microbial metabolites on the catalase activity of *Staphylococcus aureus* and its growth in vitro under peroxidation conditions.

MATERIALS AND METHODS

The microorganisms used in this work were isolated from women's genital tracts and the anterior portion of the human nose. Vaginal isolates were as follows: 21 *Lactobacillus* spp. strains, 42 *Corynebacterium* spp. strains (19 *C. minutissimum* strains, 14 *C. aqaticum* strains, and 9 *C. equi* strains), 23 *Micrococcus* spp. strains, 86 strains of coagulase-negative staphylococci (CNS) (34 *S. epidermidis* strains, 27 *S. haemolyticus* strains, 14 *S. hominis* strains, and 11 *S. capitis* strains), 14 *Escherichia coli* strains, and 12 *Staphylococcus aureus* strains. Nasal isolates included 22 *Corynebacterium* spp. strains (13 *C. minutissimum* strains and 9 *C. aqaticum* strains), 19 *Micrococcus* spp. strains, 56 strains of coagulase-negative staphylococci (27 *S. epidermidis* strains, 18 *S. haemolyticus* strains, and 11 *S. hominis* strains), and 12 *S. aureus* strains. The strain *S. aureus* ATCC 6538 P was used as the catalase producer. The isolates were identified based on the definitive classification scheme presented in Bergey's Manual [11], using the respective test systems purchased from Lachema (Czech Republic).

The effect of exogenous microbial metabolites on the catalase activity of *S. aureus* was studied as described earlier [12]. For this purpose, lactobacilli were grown in MRS broth (Sifin, Germany) for 48 h, and corynebacteria, staphylococci, micrococci, and *E. coli* were grown at 37°C in 2% nutrient broth (NPO Pitatel'nye Sredy, Makhachkala, Russia) for 24 h under microaerobic conditions (specifically, in a desiccator with a burning candle). The culture liquid (CL) was separated from bacterial cells by centrifugation at 3000 *g* for 15 min and sterilized by adding 0.2 ml chloroform to 2.5 ml CL. To prepare cell extract (CE), the bacterial cells obtained by centrifugation were washed twice with 0.9% NaCl (physiological saline solution) and resuspended in the same solution to an optical density of 0.27 (optical density was measured at 591 nm using a Dombi plate photometer and a 0.2-ml cuvette). The suspension (3 ml) was mixed with 0.2 ml chloroform. The mixture was incubated at 37°C for 60 min and centrifuged at 3000 *g* for 15 min. The supernatant represented cell extract.

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S. aureus ATCC 6538 P suspension (OD = 0.2) in 0.9% NaCl was prepared by suspending 24-h-old cells grown on an agar medium. The experimental mixture contained 0.2 ml of the *S. aureus* suspension and 0.4 ml of the CL or CE of the microorganism whose effect on the catalase activity of *S. aureus* was studied in the given experiment. Instead of the CL or CE, the control mixture contained 0.4 ml of MRS broth, nutrient broth, or 0.9% NaCl supplemented with chloroform in the amount corresponding to its concentration in the experimental mixture. After incubating at 37°C for 60 min, *S. aureus* cells were washed twice with 0.9% NaCl and resuspended in this solution to the original optical density (OD = 0.2). The catalase activity of *S. aureus* cells, both experimental and control, was measured as described earlier [12]. The results were interpreted in terms of the presence or absence of catalase inhibitors in the microorganisms tested.

One of these microorganisms (*C. minutissimum*), which inhibited the catalase activity of *S. aureus* cells to the maximal degree, was studied with respect to its ability to affect the sensitivity of *S. aureus* cells to hydrogen peroxide. To this end, 24-h-old *S. aureus* cells grown on agar medium were suspended in 0.9% NaCl to OD = 0.27 and incubated with the CL or CE of *C. minutissimum* in the same way as described in the previous paragraph. After incubating at 37°C for 60 min, the experimental cell suspensions were mixed with 0.4 ml of 0.0125, 0.125, 0.25, 0.5, and 1% H_2O_2 solutions. The control suspensions were mixed with the same volume of physiological saline. Both experimental and control suspensions were supplemented with 3 ml of fresh 2% nutrient broth and incubated for 24 h, after which the increase in the biomass of *S. aureus* was evaluated.

All experiments were performed in triplicate. The results were processed using Student's *t*-test statistics [13].

RESULTS AND DISCUSSION

Experiments showed that the CL and CE of all *Lactobacillus* spp. and *Corynebacterium* spp. strains, 89.53% of CNS, 73.91% of *Micrococcus* spp. strains, 42.86% of *E. coli* strains, and 41.67% of *S. aureus* strains isolated from women's genital tracts were inhibitory to the catalase of *S. aureus* ATCC 6538 P. In this case, the metabolites of the firsts three groups of bacteria were more inhibitory than the metabolites of the last three groups (Fig. 1). Among the nasal isolates, all *Corynebacterium* spp. strains, 98.2% of CNS, 78.9% *Micrococcus* spp. strains, and 41.5% *S. aureus* strains were found to inhibit the catalase of *S. aureus* ATCC 6538P. The most inhibitory metabolites were those of *Corynebacterium* spp. and CNS, whereas the metabolites of *Micrococcus* spp. and *S. aureus* strains were the least inhibitory (Fig. 2). The CL of the microorganisms studied inhibited catalase more severely than the CE of the same microorganisms. At the same time, we did not

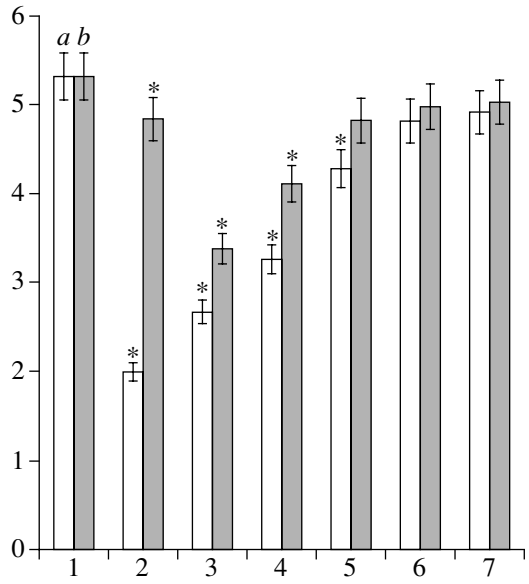

Fig. 1. The effect of (*a*) the culture liquid and (*b*) cell extract of vaginal isolates on the catalase activity of *S. aureus* ATCC 6538 P: 1, control (nutrient broth and 0.9% NaCl instead of CL and CE, respectively); 2, *Lactobacillus* spp.; 3, *Corynebacterium* spp.; 4, coagulase-negative staphylococci; 5, *Micrococcus* spp.; 6, *S. aureus* isolates; and 7, *E. coli* isolates. The ordinate shows catalase activity in μmol/(min OD unit). The asterisks mark data whose difference from the control values is statistically significant with $p < 0.05$.

reveal significant differences in the catalase-inhibiting activity of the microorganisms with respect to the type of ecotope from which they were isolated.

The results presented suggest that the microbial inhibitors of the *S. aureus* ATCC 6538 P catalase are located both inside and outside cells. In lactobacilli, catalase inhibitors are primarily extracellular. The number of catalase-inhibiting strains and their activity were greater among the dominant bacteria of the ecotopes studied (*Lactobacillus* spp., *Corynebacterium* spp., and CNS [14, 15]) than among the minor bacteria of these ecotopes (*E. coli* and *S. aureus*). These observations indicate that catalase-inhibiting activity is typical of the autochthonous microflora.

The inhibition of the *S. aureus* catalase by normal human microflora can be considered to be a mechanism of intercellular interactions responsible for the formation and/or stabilization of microbiocenoses due to suppression of the self-defense systems of catalase-positive microorganisms, both allo- and autochthonous. The inhibition of the antioxidant activity of microorganisms promotes the detrimental effect of active oxygen species and may influence the survival and growth of these microorganisms in microbiocenoses. This suggestion is confirmed by the fact that intra- and extracellular microbial metabolites, except for those of *Lactobacillus* spp., do not directly affect the growth characteristics of staphylococci but, as is evident from the

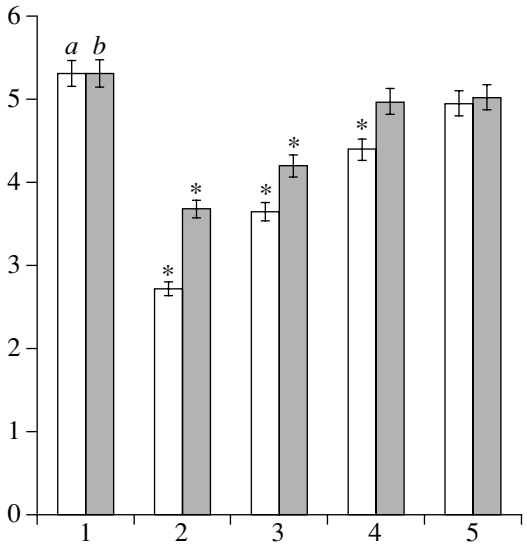

Fig. 2. The effect of (*a*) the culture liquid and (*b*) cell extract of nasal isolates on the catalase activity of *S. aureus* ATCC 6538P: 1, control (nutrient broth and 0.9% NaCl instead of CL and CE, respectively); 2, *Corynebacterium* spp.; 3, coagulase-negative staphylococci; 4, *Micrococcus* spp.; and 5, *S. aureus* isolates. The ordinate shows catalase activity in μmol/(min OD unit). The asterisks mark data whose difference from the control values is statistically significant with $p < 0.05$.

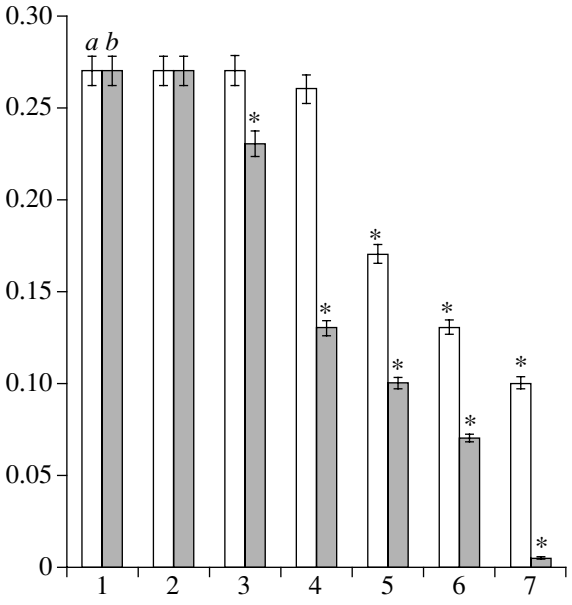

Fig. 3. The effect of hydrogen peroxide on the growth of (*a*) untreated *S. aureus* ATCC 6538 P cells and (*b*) those pretreated with the extracellular metabolites of *Corynebacterium minutissimum*: 1, control (0% H_2O_2); 2, control (with the addition of the medium used for the growth of *C. minutissimum*); 3, 0.0125% H_2O_2; 4, 0.125% H_2O_2; 5, 0.25% H_2O_2; 6, 0.5% H_2O_2; and 7, 1% H_2O_2. The ordinate shows the optical density of broth culture in OD units. The asterisks mark data whose difference from the control values is statistically significant with $p < 0.05$.

experiments on the effect of the CL of *C. minutissimum* and hydrogen peroxide on the growth of *S. aureus* ATCC 6538 P, enhance the susceptibility of staphylococci to hydrogen peroxide (Fig. 3). Different effects of corynebacterial metabolites on bacterial growth and catalase activity suggest that the inhibition of the antioxidant systems of microorganisms may occur without influencing their growth.

Thus, autochthonous microorganisms, which determine the state of microbiocenoses, influence other species of these cenoses not only through antagonistic activity but also through their action on the enzymatic systems of these species. The inhibitory effect of intra- and extracellular microbial metabolites on *S. aureus* catalase can be considered to be a mechanism of intercellular interactions responsible for the formation of microbiocenoses.

REFERENCES

1. Bukharin, O.V., *Persistentsiya patogennykh bakterii* (Persistence of Pathogenic Bacteria), Moscow: Meditsyna, 1999.
2. Pahlson, C. and Larsson, P.G., The Ecologically Wrong Vaginal Lactobacilli, Med. Hypotheses, 1991, vol. 36, pp. 125–130.
3. Usvyatsov, B.Ya., Lysozyme and Antilysozyme Activities of Pathogenic Cocci, *Doctoral (Med.) Dissertation*, Leningrad, 1986.
4. Mitrokhin, S.D. and Ivanov, A.A., Significance of Fecal Carbonic Acids in the Diagnosis of Dysbacteriosis, *Mikrobiol. Zh.*, 1995, no. 4, pp. 99–101.
5. Egorov, N.S. and Baranova, I.P., Bacteriocins: Formation, Properties, and Applications, *Antibiot. Khimioter.*, 1999, vol. 44, no. 6, pp. 33–40.
6. Hillier, S.L., Krohn, M.A., Klebanoff, S.J., and Eschenbach, D.A., The Relationship of Hydrogen Peroxide–producing Lactobacilli to Bacterial Vaginosis and Genital Microflora in Pregnant Women, *Obstet. Gynecol.*, 1992, vol. 79, no. 3, pp. 369–373.
7. Smirnova, G.V., Muzyka, N.G., Glukhovchenko, M.N., and Oktyabr'skii, O.N., Hydrogen Peroxide Modulates the Intracellular Levels of Thiols and Potassium in *Escherichia coli* Cells, *Mikrobiologiya*, 1998, vol. 67, no. 5, pp. 594–600.
8. Menendez, M.C., Ainsa, J.A., Martin, C., and Garcia, M.J., *katGI* and *katGII* Encode Two Different Catalases–Peroxidases in *Mycobacterium fortuitum*, *J. Bacteriol.*, 1997, vol. 179(22), pp. 6880–6886.
9. Mobley, H.L., *Helicobacter pylori* Factors Associated with Disease Development, *Gastroenterology*, 1997, vol. 113(6), pp. 21–28.
10. Zemser, R.B. and Martin, S.E., Heat Stability of Virulence-associated Enzymes from *Listeria monocytogenes* SLCC 5764, *J. Food Prot.*, 1998, vol. 61(7), pp. 899–902.
11. *Bergey's Manual of Systematic Bacteriology*, 9th ed., Holt, J.G. *et al.*, Eds., Baltimore: Williams & Wilkins,

1994. Translated under the title *Opredelitel' bakterii Berdzhi*, Moscow: Mir, 1997.

12. Bukharin, O.V., Cherkasov, S.V., Sgibnev, A.V., Zabirova, T.M., and Ivanov, Yu.B., The Effect of Microbial Metabolites on the Catalase Activity and Growth of *Staphylococcus aureus* 6538 P, *Byull. Eksp. Biol.*, 2000, vol. 130, no. 7, pp. 80–82.

13. Ashmarin, I.P. and Vorob'ev, A.A., *Statisticheskie metody v mikrobiologicheskikh issledovaniyakh* (Statistical Methods in Microbiological Investigations), Leningrad, 1962.

14. Parshuta, L.I., Formation of Nasal Microbial Cenoses in Staphylococcus Carriers, *Cand. Sci. (Med.) Dissertation*, Orenburg, 1998.

15. Sokolova, K.Ya., Savel'eva, I.V., Popova, I.V., *et al.*, *Autoflora cheloveka v norme i patologii i ee korrektsiya* (Autogenous Human Microflora in Norm and Pathology and Its Correction), Blokhina, I.N., Ed., Gor'kii, 1998, pp. 144–148.