Effects of Microbial Metabolites on Catalase Activity and Growth of *Staphylococcus aureus* 6538 P

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The effects of cell extracts and supernatants of *Lactobacillus spp.* and *Corynebacterium spp.* on catalase activity and growth of *Staphylococcus aureus* 6538 P were studied. Intra- and extracellular metabolites of lactobacilli and corynebacteria inhibited catalase activity of *S. aureus* 6538 P. The growth of *S. aureus* 6538 P decreased after incubation with lactobacillus metabolites. The inhibitory effect of intra- and extracellular metabolites of lactobacilli and corynebacteria on catalase activity of *S. aureus* is a possible pathway of microbial interrelations responsible for the formation and/or development of microbial biocenoses.

Key Words: *lactobacilli; corynebacteria; S. aureus; catalase; microbial inhibitors; bacterial growth*

Bacterial interrelations play a role in the formation of microbial biocenoses [5]. It was shown that H_2O_2-producing lactobacilli are involved in the maintenance of organisms resistance to various pathogens [3]. Microbicidal effects of H_2O_2 are inhibited by microbial catalase enabling the persistence of catalase-positive bacteria under conditions of peroxidation [1]. However, it is still unclear whether normal microbial flora produces inhibitors of bacterial catalase. The solution of this problem will provide new insights in the mechanisms of microbial interrelations contributing to the maintenance of colony resistance and formation of stable microbial biocenoses.

Here we studied the effects of metabolites of lactobacilli and corynebacteria on catalase activity (CA) and growth of *S. aureus* 6538 P.

MATERIALS AND METHODS

Lactobacillus spp. (10 strains) and *Corynebacterium spp.* (10 strains) were isolated from reproductive organs of women. *S. aureus* ATCC 6538 P was used as catalase producer.

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To measure CA of *S. aureus* freshly prepared 0.0125 M H_2O_2 (1 ml) was added to 0.2 ml *S. aureus* suspension adjusted to optical density (OD) of 0.2 rel. units and incubated at room temperature for 10 min. The reaction of H_2O_2 degradation catalyzed by catalase was stopped by the addition of 5 drops of 2 N HCl. Freshly prepared 0.025 M KCl (1 ml) was added, the solution was thoroughly mixed, and *S. aureus* cells were precipitated by centrifugation at 3000*g* for 15 min. Light absorption of I_2-KI complex in the supernatant was measured in a 0.2-ml cuvette at 492 nm not later then 10 min after centrifugation.

CA was calculated per OD unit by the formula:

$$12.5\times(1-OD_E/OD_C)/T\times OD_M,$$

where OD_E and OD_C are OD of I_2-KI complex in the experiment and control (without incubation with bacteria), respectively; T is the time of incubation with H_2O_2; and OD_M is OD of bacterial suspension.

Lactobacilli were cultured in MRS broth (Sifin) for 48 h; corynebacteria were cultured in 2% meat-peptone broth (MPB, Research-and-Production Corporation Pitatel'nye Sredy, Makhachkala) at 37°C for 24 h under aerobic conditions. Supernatants were isolated from bacterial cells by centrifugation at 3000g for 15 min and sterilized with 0.2 ml chloroform. To

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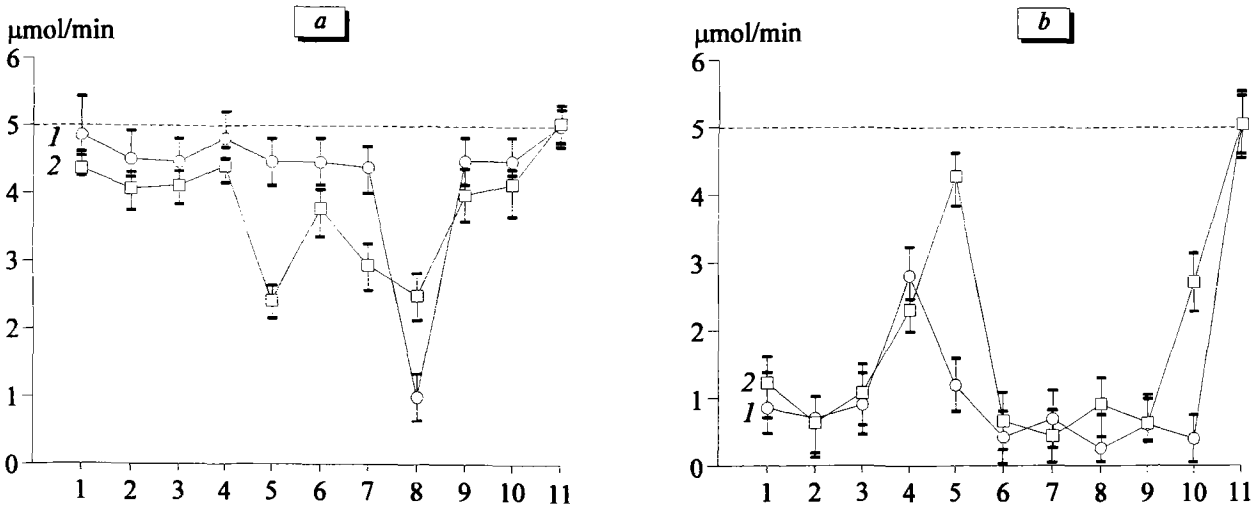

Fig. 1. Effects of metabolites of corynebacteria (*a*) and lactobacilli (*b*) on *S. aureus* catalase activity. Abscissa: microbial strains. Ordinate: catalase activity of *S. aureus* per optical density unit. Here and in Fig. 2: dotted line: control; supernatants (*1*); cell extracts (*2*).

obtain cell extracts, microbial cells were washed 2 times and resuspended in 0.9% NaCl. Chloroform (0.2 ml) was added to 3 ml microbial suspension, and the mixture was incubated at 37°C for 60 min and then centrifuged at 3000*g* for 15 min. The supernatant was used for further analyses.

MRS broth, MPB, and 0.9% NaCl treated with chloroform served as the control. Bacterial suspension (5×10^6 colony-forming units/ml) was prepared from 1-day-old culture of *S. aureus*. Supernatants or bacterial cell extracts (0.4 ml) were added to 0.2 ml *S. aureus* suspension; in the control, MRS broth, MPB, or 0.9% NaCl treated with chloroform were used. The mixture was incubated at 37°C for 60 min and washed 2 times, the suspension of *S. aureus* cell was adjusted to OD 0.2 rel. units (591 nm), and CA was measured. The growth of *S. aureus* was evaluated from OD of the suspension after 24-h culturing in broth. To this end, 2% MPB (3 ml) was added to washed and standardized suspensions of *S. aureus* (0.2 ml).

The results were analyzed by the method described elsewhere [2].

RESULTS

Cell extracts and supernatants of lactobacilli and corynebacteria inhibited CA of *S. aureus* 6538 P (Fig. 1). Supernatants of lactobacilli more potently inhibited CA than cell extracts (0.88±0.07 *vs.* 1.18±0.07 μmol/min, $p<0.001$). By contrast, cell extracts of corynebacteria were more potent than supernatants in inhibiting CA (3.67±0.06 *vs.* 4.52±0.05 μmol/min). There was a strong correlation between the inhibitory effects of cell extracts and supernatants of broth cultures (r=0.90-0.93).

Supernatants and, to a lesser degree, cell extracts of lactobacilli inhibited the growth of *S. aureus* (Fig. 2), while metabolites of corynebacteria did not affect this parameter. There was no correlation between the effects of cell extracts and supernatants of lactobacilli (r=0.24 and r=0.34, respectively) and corynebacteria (r=0.19 and r=0.2, respectively) on the growth and CA of *S. aureus*.

Thus, bacterial cell extracts and supernatants of broth cultures inhibited CA of *S. aureus* 6538 P. These data indirectly indicate various localizations (intra- and extracellular) of microbial inhibitors of bacterial catalase. Different effects of bacterial metabolites on the growth and CA of *S. aureus* and the absence of correlation between these parameters suggest that microbial metabolites regulate the growth and oxidative processes in *S. aureus* by various mechanisms. Therefore, enzymatic processes in microbial biocenoses are regulated by various mechanisms [4].

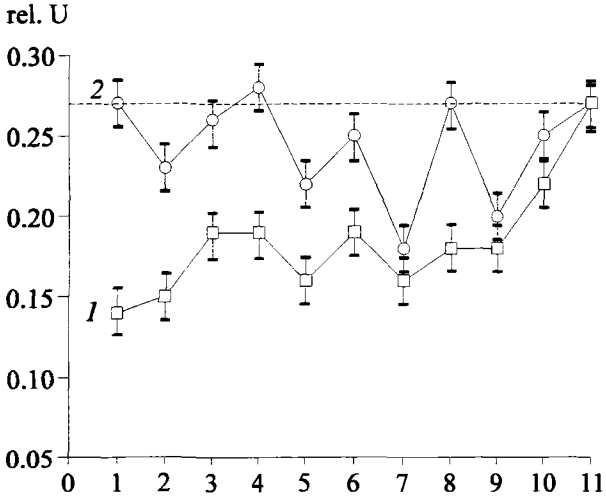

Fig. 2. Effects of lactobacillus metabolites on growth of *S. aureus*. Abscissa: microbial strains. Ordinate: optical density of *S. aureus* broth culture.

It should be emphasized that microbial inhibitors of catalase can function as autoregulators of endogenous oxidative processes in bacteria. The inhibition of CA by metabolites of normal microbial flora is probably a mechanism of microbial interrelations responsible for the formation and/or stabilization of microbial biocenoses. In this case, various defense systems of catalase-positive bacteria protecting them form toxic effects of H_2O_2 produced by normal microbial flora are inhibited.

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