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ELIMINATION OF CEFTRIAXONE-INDUCED HORMESIS IN *STAPHYLOCOCCUS AUREUS* BY METABOLITES OF PROBIOTIC BACTERIA OF THE GENUS *BACILLUS*

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Abstract. The study investigates the elimination of ceftriaxone-induced hormesis in *Staphylococcus aureus* 209 using probiotic metabolites. Subinhibitory concentrations of ceftriaxone (0.125–0.0075 μg/mL) were found to stimulate pathogen growth, significantly shortening the lag phase. To counteract this effect, the cell-free culture supernatant (CFCS) of the probiotic "Biosporin" (*Bacillus subtilis* and *B. licheniformis*) was applied. The combined use of ceftriaxone and bacillary CFCS completely neutralized the hormetic effect, ensuring 100 % inhibition of *S. aureus* viability across all tested subinhibitory points. Thus, *Bacillus* cell-free metabolites represent promising enhancers for overcoming antibiotic-induced bacterial stress.
Keywords: *Staphylococcus aureus*, *Bacillus* spp., ceftriaxone, subinhibitory concentrations, hormesis, elimination.

Subinhibitory antibiotic concentrations can paradoxically trigger hormesis and enhance virulence in *Staphylococcus aureus* (Müller, 2020; Prithviraj, 2021; Vargas-Cruz, 2022). Combining traditional antibiotics with cell-free metabolites from *Bacillus* probiotics offers a promising strategy to neutralize this stimulatory effect and combat bacterial resistance (Byun, 2023, Mazanko, 2026). This work aims to evaluate the efficacy of probiotic *Bacillus* metabolites in eliminating ceftriaxone-induced hormesis in *S. aureus*. You can review the complete research overview and proposal details.

Materials and Methods. The test strain *Staphylococcus aureus* 209 was used. Primary screening of the antagonistic potential of 7 *Bacillus* strains was performed via the deferred antagonism method on susceptibility agar (n = 3). Based on the results, the commercial probiotic "Biosporin" (LLC "PHC "Biopharma", Ukraine; *B. subtilis* UKM B-5007 and *B. licheniformis* B-5514) was selected. Its cell-free culture supernatant (CFCS) was obtained by cultivation in meat-peptone broth (MPB) for 48 h (37 °C), centrifugation, and filtration through 0.22 μm filters (Millipore).

The MIC of ceftriaxone and *S. aureus* growth kinetics were determined by serial microdilution in 96-well plates. The antibiotic range was 1.0–0.0075 μg/mL, the initial inoculum was $5 \cdot 10^5$ CFU/mL, and the final volume was 200 μL. Kinetics were monitored automatically using a microplate reader (OD_{560}, 48 h, 1-h interval, 37 °C). To evaluate the intrinsic effect of CFCS (dilutions 1:1–1:256) and the "ceftriaxone + CFCS" combination (final working dilution 1:1), double-strength MPB (2 x MPB) was used to maintain a standard

nutrient concentration. The control row contained equivalent volumes of 0.5 % NaCl. Viable pathogen cells after 24 h of co-cultivation were quantified by the Gould method, with results expressed as lg CFU/mL).

Results. The MIC of ceftriaxone against the *S. aureus* 209 test strain was determined to be 0.25 μg/mL. Under the influence of subinhibitory concentrations of the antibiotic (0.125–0.0075 μg/mL), a pronounced hormetic effect was observed. This phenomenon was manifested by a significant reduction in the lag phase: intensive exponential growth of the staphylococcal culture in the experimental wells initiated as early as 4–6 h of cultivation, compared to 24 h in the growth control. By 48 h, the optical density of the antibiotic-stressed cells (OD_{560} = 0.48–0.54) significantly exceeded that of the control (OD_{560} = 0.41), demonstrating profound metabolic stimulation of the bacterial population by low cephalosporin doses

In the first stage, a comparative assessment of the antagonistic activity of *Bacillus* strains was conducted. Among the commercial probiotic preparations, "Biosporin" (*B. subtilis* UKM B-5007 + *B. licheniformis* B-5514) demonstrated the highest efficacy against the *S. aureus* 209 test strain, with a growth inhibition zone of 19 ± 2.6 mm, whereas for "Subalin" and "Enterogermina" this parameter was only 14 ± 0.7 mm and 6 ± 3.2 mm, respectively. Given its high anti-staphylococcal potential, the cell-free culture supernatant (CFCS) of "Biosporin" was selected for further modeling of the antibiotic-induced hormesis elimination system.

When evaluating the intrinsic antimicrobial activity of various CFCS dilutions of "Biosporin", complete inhibition of *S. aureus* 209 growth (growth control – 8 lg CFU/mL was observed within the dilution range of 1:1 to 1:4. Starting from a 1:8 dilution, cell viability recovered 3 lg CFU/mL, gradually approaching the initial control values. The study of the combined effect confirmed the presence of antibiotic-induced hormesis: under subinhibitory concentrations of ceftriaxone (0.125–0.0075 μg/mL), the number of viable pathogen cells increased to 8.7–10 lg CFU/mL compared to the control. The maximum stimulatory effect was recorded at an antibiotic concentration of 0.015 μg/mL. The introduction of native "Biosporin" CFCS into the test system (final working dilution 1:1) resulted in the complete eradication of *S. aureus* 209 viability across the entire range of ceftriaxone concentrations. Thus, the complex of extracellular bacillary metabolites completely eliminated the hormetic growth surge of the staphylococcal culture induced by subinhibitory cephalosporin doses.

Conclusions. Subinhibitory concentrations of ceftriaxone induce a pronounced hormetic effect in *Staphylococcus aureus*, significantly accelerating its growth and increasing the final population titer compared to the control. The cell-free culture supernatant (CFCS) of the probiotic "Biosporin" exhibits high intrinsic antimicrobial activity against the staphylococcal culture, which is maintained even under significant dilution of the metabolites. The combined application of ceftriaxone and bacillary cell-free metabolites results in the complete elimination of antibiotic-induced hormesis and 100 % inhibition of pathogen viability, opening new avenues for optimizing antimicrobial therapy.

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ACTIVITY OF CURCUMIN METAL COMPLEXES AGAINST MATURE *PSEUDOMONAS AERUGINOSA* BIOFILMS

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Abstract. The aim of this study was to evaluate the antibiofilm activity of curcumin complexes with zinc, neodymium, ytterbium, and dysprosium against mature biofilms formed by *Pseudomonas aeruginosa* strains. The results demonstrated that the curcumin metal complexes had antibiofilm activity against mature biofilms formed by some *Pseudomonas aeruginosa* strains.
Keywords: *Pseudomonas aeruginosa,* curcumin, biofilm, antibiofilm activity, metal–curcumin complexes

Biofilm formation is an important stage in the development of many chronic infections, with the formation of the extracellular matrix being a key factor in this process. Together with the metabolic reprogramming of cells, the extracellular matrix provides protection to microorganisms (Osondu-Chuka et al., 2026). During biofilm maturation, cells form microcolonies and secrete an extracellular matrix, which may account for more than 90 % of the biofilm mass. Matrix components can bind antimicrobial compounds or promote their enzymatic degradation, thereby reducing the concentration of antimicrobial agents reaching bacterial cells. Thus, the matrix acts as a functionally active environment that maintains biofilm integrity and protects bacteria from environmental factors, particularly antimicrobial agents (Flemming et al., 2025). Given the high tolerance of mature biofilms to antimicrobial agents, the search for compounds capable of affecting their functioning is of particular relevance. Curcumin is one promising natural compound, and its metal-modified derivatives have been reported to exhibit antibacterial and antibiofilm activity against *P. aeruginosa* (Shariati et al., 2024; Mittal et al., 2024).

The antibiofilm activity of curcumin complexes with zinc, ytterbium, neodymium, and dysprosium was evaluated against strains of *P. aeruginosa* ATCC 15692, ATCC 10145, and ATCC 9027. Mature biofilms were formed for 48 h in polystyrene microplates, after which the studied compounds were added to the culture medium. Following an additional 24 h of incubation, biofilm biomass was determined spectrophotometrically using crystal violet staining (Haney et al., 2022).

None of the studied compounds exhibited antibiofilm activity against *P. aeruginosa* ATCC 15692 or ATCC 10145. In contrast, a reduction in biofilm biomass was observed for *P. aeruginosa* ATCC 9027. The most pronounced inhibitory effect was observed for the dysprosium complex, which reduced biofilm biomass by 32 % at 25 μM and 37 % at 50 μM