SECTION 7.
BIOLOGY AND BIOTECHNOLOGY

Bodiul Anastasiia
1st year master's student at the Faculty of TVPPTSB
Mykolaiv National Agrarian University, Ukraine

Supervisor: Karatieieva Olena
Ph.D., docent, docent of the Department of Biotechnology and Bioengineering
Mykolaiv National Agrarian University, Ukraine

EFFECT OF CONCENTRATIONS ANTIBIOTIC STANDARD ON THE INHIBITION ZONES OF TEST CULTURES

Antibiotics are biologically active substances that have the ability to destroy or inhibit the development of various microorganisms, including bacteria. They can be formed naturally in the course of the life of certain microorganisms, such as fungi or bacteria, or be synthesized artificially in the laboratory [3].

Concerns about the possible impact of residual amounts of antibiotics in the environment on human health are primarily driven by two main factors. First, the release of antibiotic residues into the environment can alter the human microbiome, creating favorable conditions for the selection and spread of antibiotic-resistant bacteria that live in the human body. This can significantly complicate the treatment of infections and contribute to the growth of antibiotic resistance [2].

Secondly, there is a danger that residual antibiotics create selection pressure on microorganisms inhabiting the environment, contributing to the formation of antibiotic resistance reservoirs in natural ecosystems. This can lead to the further spread of antibiotic-resistant bacteria, which can subsequently enter the human body through water, food, or direct contact with contaminated environments [1-2].

The test cultures *Bacillus mycoides ATCC 537*, *Micrococcus flavus ATCC 10240*, *Kocuria rhizophila ATCC 9341* were selected for the study due to their specific susceptibility to antibiotics of the streptomycin, zincbacitracin, and penicillin groups. The relevance of studying these groups of antibiotics is due to the widespread use of penicillin antibiotics in the food industry, as well as the active use of streptomycin and zincbacitracin in the animal husbandry industry, which contributes to their accumulation in the environment [4].

Based on this, the aim of the study was to investigate the effect of the concentrations of reference antibiotics on the zones of growth inhibition of the above antibiotic strains.

The study was conducted at the Mykolaiv Regional State Laboratory of the State Service of Ukraine for Food Safety and Consumer Protection (MRSFSCP). The Testing Laboratory accredited by the National Academy of Agricultural Sciences of Ukraine in accordance with the requirements of DSTU ISO/IEC 17025:2017 was implemented on the basis of MRSFSCP [5].

Determination of the relationship between the concentration of the antimicrobial agent and the size of the inhibition zone allowed us to establish thresholds at which complete or partial inhibition of microbial growth is observed. The concentrations of the working solutions of the reference antibiotics should show zones of inhibition of the test culture of microorganisms with a diameter of about 17±1 mm.

For an objective comparison of concentrations, a comparison was made: one of the dilutions was 10 μg/cm³, while the others had values equal to or not exceeding 2 μg/cm³. However, in the case of zincbacitracin, concentrations of ≤ 2 μg/cm³ were compared.

In accordance with the stated purpose of the study, as well as taking into account the specifics of the dilutions of the reference standards of antibiotics, their values are shown in Table 1.

Table 1

Dilution of reference antibiotics to study the effect of different concentrations on the zones of growth inhibition of test cultures

Name of the reference antibiotic	Dilution number	Amount of diluted antibiotic	Amount, No. of buffer	Obtained concentration, μg/cm³
Streptomycin sulfate	2	0,1 cm³ No. 1	9,9 cm³ No. 6	10
	3	2 cm³ No. 2	8 cm³ No. 6	2
Benzylpenicillin sodium salt	2	0,1 cm³ No. 1	9,9 cm³ No. 4	10
	4	5 cm³ No. 3	5 cm³ No. 4	0,05
Zincbacitracin	3	2 cm³ No. 2	8 cm³ No. 2	2
	4	1 cm³ No. 3	9 cm³ No. 2	0,2

The results were evaluated and interpreted by measuring the zones of growth inhibition of test cultures using high-precision measuring instruments.

For visual familiarization with the results of the experiment, a linear histogram (Fig. 1) shows the dependence of the size of the inhibition zones on the concentration of antibiotics for the three test cultures: *Bac. mycoides ATCC 537, K.*

rhizophila ATCC 9341 and *Mic. flavus ATCC 10240.*

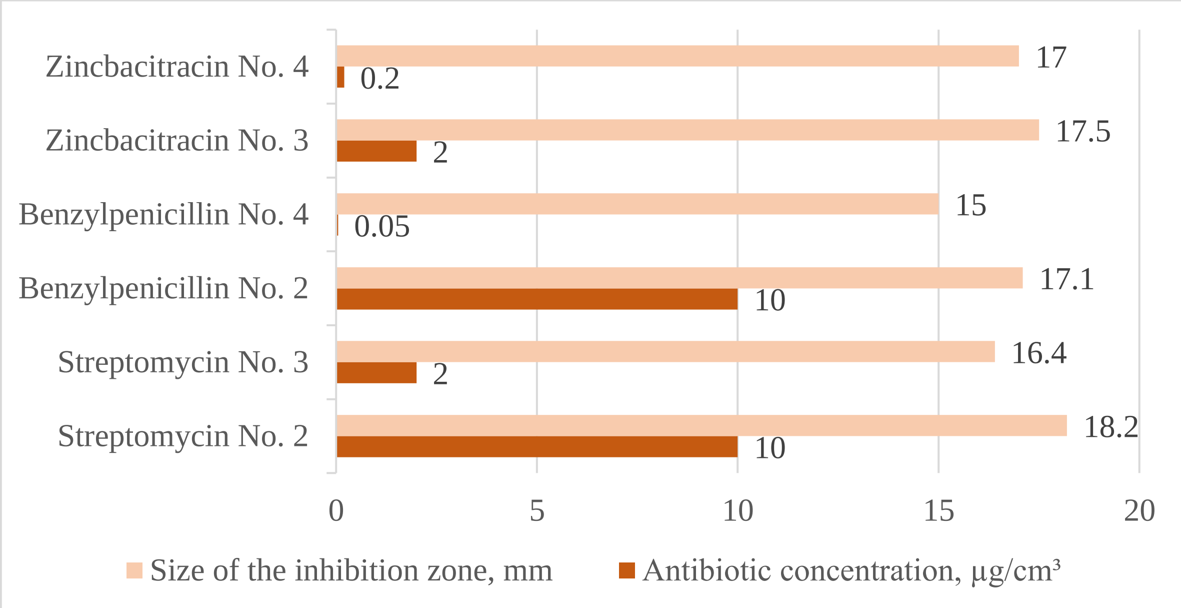

Fig. 1. **Dependence of the formation of growth inhibition zones on the concentration of the reference antibiotic**

Accordingly, we determined the variability of the size of the inhibition zones and their morphological characteristics depending on the antibiotic concentration. The results of the effect of concentrations on the formation and size of inhibition zones are presented in Table 2. For *Bac. mycoides ATCC 537*, under the influence of streptomycin sulfate at a concentration of 10 µg/cm³, the inhibition zone reached 18.2 mm, had clear contours and single white inclusions. When the concentration was reduced to 2 µg/cm³, the inhibition zone decreased to 16.4 mm, and its edges became blurred, with a characteristic "circle-shaped" delamination. The zones from both concentrations are acceptable within the standard value of the size of the zones 17±1 mm and their morphological parameters.

Table 2

Analysis of the formation of zones of inhibition at different concentrations of antibacterial drugs

Test culture strain	Reference antibiotic	Dilution concentration value, µg/cm³	Size of inhibition zones, mm	Nature of the formed zones
Bac. mycoides ATCC 537	Streptomycin sulfate	10	18.2	Clear, with single white inclusions
		2	16.4	Blurred edges, visible "circles" of stratified zones

Table continuation 2

K. rhizophila ATCC 9341	Benzylpenicillin	10	17.1	Partially blurred edges, no inclusions
		0.05	15	Blurred, without inclusions
Mic. flavus ATCC 10240	Zincbacitracin	2	17.5	Clear, without inclusions and traces of delamination
		0.2	17	Partially blurred, with single white inclusions

Source: based on own research

In the case of *K. rhizophila ATCC 9341*, benzylpenicillin at a concentration of 10 μg/cm³ formed an inhibition zone with a diameter of 17.1 mm with partially blurred edges without visible inclusions. When the concentration was reduced to 0.05 μg/cm³, the diameter of the inhibition zone decreased to 15 mm, and the edges became even more blurred, which may indicate a decrease in the effectiveness of the antibiotic. The inhibition zone with a concentration of 0.05 μg/cm³ does not meet the standard indicators, and therefore is ineffective in this study.

For *Mic. flavus ATCC 10240* under the influence of zincbacitracin at a concentration of 2 μg/cm³, the inhibition zone reached 17.5 mm, had clear edges without inclusions, but traces of delamination were observed. When the concentration was reduced to 0.2 μg/cm³, the inhibition zone was slightly smaller and was characterized by partial blurring of the edges and the presence of single white inclusions. As in the case of *ATCC 537*, the inhibition zones for both concentrations correspond to the standard range of 17±1 mm and satisfy their morphological criteria.

Conclusions: To summarize, we found that the size and morphological characteristics of the inhibition zones of the test cultures depend on the concentration of the antibiotic used. Higher concentrations contributed to the formation of clearer inhibition zones, while a decrease in concentration led to a decrease in the effectiveness of the antibiotic, which was manifested by a decrease in the diameter of the zones, blurring of their edges and the appearance of inclusions. The most stable results were observed when streptomycin sulfate (10 μg/cm³) was used for *ATCC 537* and benzylpenicillin (10 μg/cm³) for *ATCC 9341*. The obtained results indicate the variability of microorganisms' susceptibility to antibiotics depending on their concentration, which is associated with the mechanisms of drug penetration into bacterial cells or adaptive reactions of microorganisms.

References:

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