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BACTERICIDAL AND FUNGICIDAL ACTIVITY OF SILVER NANOPARTICLES STABILIZED BY DIDECYLDIMETHYLAMMONIUM BROMIDE

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Abstract

As is known, silver nanoparticles have unique physical, chemical and medico-biological properties, which make it possible to apply them in various fields of medicine, food industry, sanitation and other branches of science and technology. Silver nanoparticles exert bactericidal, bacteriostatic, antiviral and antifungal properties against more than 500 pathogens, yeast fungi and viruses. The prospect of using nanosilver as a disinfecting agent is due to its following advantages: no adaptation of microorganisms, safety in relation to the human body, universality of action, high antimicrobial effect. Within the framework of the present work, the bactericidal and fungicidal properties of nanosilver stabilized by a quaternary ammonium compound - didecyldimethylammonium bromide were investigated.

In order to study antimicrobial activity and determine the minimum inhibitory concentration, tests on the sensitivity of microorganisms to the developed silver nanoparticles by serial dilutions on a liquid nutrient medium were carried out. As a bacterial culture, *Escherichia coli* was used. Meat and peptone broth was used as a nutrient medium for the cultivation of test strains. Test tubes with *E. coli* cultures were placed in a thermostat for 24 hours at a temperature t = 37 ^{0}C. After a day, the changes that occurred in test tubes were registered. The fungicidal activity of nanosized silver stabilized by didecyldimethylammonium bromide was studied on a *Penicillium roqueforti* mold culture, using the disk diffusion method. Sowing the spore suspension of fungi was carried out on the surface of the nutrient medium - Saburo agar. Next, paper disks with a diameter of 10 mm were placed on the prepared culture sample, each of which was coated with the same amount of silver nanoparticles preparation with different concentrations: C (Ag) = 0.5 mg/mL, 0.05 mg/mL, 0.005 mg/mL, and 0.0005 mg/mL. Then the samples were incubated in a thermostat at a temperature of t = 25 ^{0}C; after two days, the growth suppression zone of mold culture *Penicillium roqueforti* was analyzed. Another experiment was also conducted to determine the effect of the active acidity of the medium on the fungicidal activity of the developed nanosilver preparation. In this experiment, a series of samples with various pH values from 3 to 11 was prepared. The studies were also performed using the disk diffusion methods with Saburo agar as a medium.

It was found that the minimum inhibitory concentration of nanosilver stabilized by didecyldimethylammonium bromide with respect to *E. coli* is 0.005 mg/mL. After analyzing the experimental data obtained, we can talk about the synergistic antibacterial effect of silver nanoparticles and stabilizer - didecyldimethylammonium bromide. Regarding the mold culture of *Penicillium roqueforti*, it was determined that samples with concentrations of the disinfecting component C (Ag) = 0.5 mg/mL, 0.05 mg/mL, 0.005 mg/mL significantly inhibit the vital activity of the mold culture.

It has been established that in the entire range of active acidity of the medium (pH) under consideration, nanosilver stabilized by DDAB exhibits high fungicidal activity.

***Key words**: Nanosilver, Didecyldimethylammonium bromide, Fungicidal and bactericidal activity.*

1. Introduction

As is known, silver nanoparticles have unique physical, chemical and medico-biological properties, which make it possible to apply them in various fields of: medicine, food industry, sanitation and other branches of science and technology. Silver nanoparticles exert: bactericidal, bacteriostatic, antiviral and antifungal properties against more than 500 pathogens, yeasts, fungi and viruses [1 - 8].

Also, in many countries, quaternary ammonium compounds (QAC) are increasingly used as bactericidal substances. Modern QACs are characterized by a moderately wide spectrum of antimicrobial activity. They are: odorless, colorless, resistant to high temperatures, they have low corrosiveness, high stability of concentrates and dilute solutions, relative tolerance to the presence of organic substances, residual bacteriostatic effect on treated surfaces, low toxicity, and excellent detergents properties [9 - 12].

In this regard, it is urgent to create a complex preparation based on silver nanoparticles stabilized with a quaternary ammonium compound - didecyldimethylammonium bromide and study its bactericidal and fungicidal activity.

2. Materials and Methods

The preparation procedure, properties and structure of silver nanoparticles stabilized by didecyldimethylammonium bromide are presented in the works of Barabanov *et al.*, [13], and Blinov *et.al.*, [14].

In order to study antimicrobial activity and determine the minimum inhibitory concentration, tests on the sensitivity of microorganisms to the developed silver nanoparticles by serial dilutions on a liquid nutrient medium were carried out. As a bacterial culture, *Escherichia coli* was used. Peptone meat broth was used as a nutrient medium for the cultivation of test strains. Test tubes with *E. coli* cultures were placed in a thermostat for 24 hours at a temperature t = 37 ^{0}C. After a day, the changes that occurred in test tubes were registered.

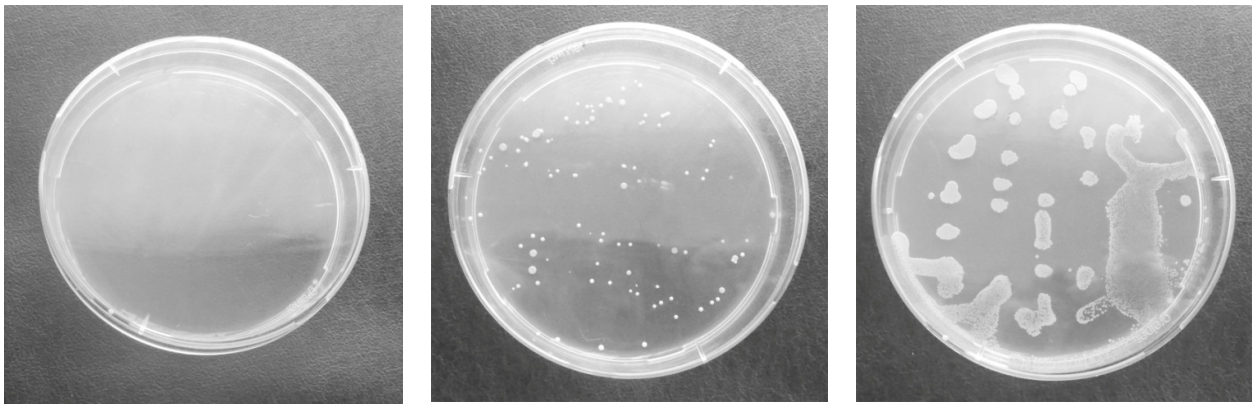
The fungicidal activity of nanosized silver stabilized by didecyldimethylammonium bromide (DDAB) was studied on a *Penicillium roqueforti* mold culture, using the disk diffusion method. Sowing the spore suspension of fungi was carried out on the surface of the nutrient medium - Saburo agar. Next, paper disks with a diameter of 10 mm were placed on the prepared culture sample, each of which was coated with the same amount of silver nanoparticles preparation with different concentrations: C (Ag) = 0.5 mg/mL, 0.05 mg/mL, 0.005 mg/mL, and 0.0005 mg/mL. Then the samples were incubated in a thermostat at a temperature of t = 25 ^{0}C; after two days, the growth suppression zone of mold culture *Penicillium roqueforti* was analyzed by measuring the diameters of the suppression zones (in millimeters) around the discs using a ruler (Vernier caliper). Due to the process of diffusion of the nanosilver preparation into the nutrient medium, a zone of inhibition of the growth of microorganisms around the disks is formed.

Another experiment was also conducted to determine the effect of the active acidity of the medium on the fungicidal activity of the developed nanosilver preparation. In this experiment, a series of samples with various pH values from 3 to 11 was prepared. The required value of the active acidity of the medium in the nanosilver samples was formed by adding a certain amount of NaOH (1M) and HCl (1M) solutions. The active acidity of the medium was measured using an Expert-001 pH meter-ionomer (RPC "Ekoniks-Expert", Russia). The studies were also performed by disco-diffusion methods using Saburo agar as a medium.

3. Results and Discussion

The results of determining the bactericidal activity of nanosilver stabilized by DDAB are presented in Figure 1.

It was established that at a concentration of nanosilver, C (Ag) = 0.005 mg/mL, there was no growth of microorganisms in the nutrient medium; at concentration C (Ag) = 0.0005 mg/mL - an insignificant growth of *Escherichia coli* microorganisms was observed; and at a concentration of nanosilver C = 0.00005 mg/mL, an intensive growth of bacterial colonies was observed. The smallest amount of the nanosilver preparation, giving visually complete microorganisms growth reduction (clear broth), corresponds to the minimum inhibitory concentration, i.e. the minimum inhibitory concentration (MIC) of the developed preparation is 0.005 mg/mL.



a b c

Figure 1. Photos of Petri dishes with *Escherichia coli* culture with different concentrations of nanosilver preparation: a - 0.005 mg/mL; b - 0.0005 mg/mL; c - 0.00005 mg/mL

In order to evaluate the antimicrobial activity of the developed complex preparation of nanosilver, a comparative analysis of its MIC with a MICs of other antibacterial agents was carried out, namely, such as: silver ions; silver nanoparticles, stabilized by a substance lacking antimicrobial activity (citrate ion), and didecyldimethylammonium bromide [15]. The values of the MICs of the above substances are shown in Table 1.

Table 1. Minimum inhibitory concentrations of substances

Substance	MIC, μg/mL
Ag^+	10
Nanosilver-citrate ion	10
Didecyldimethylammonium bromide	≈ 2000
Complex preparation	
Nanosilver	5
DDAB	2.4

Analysis of the data presented in Table 1 showed that the MIC of colloidal silver and didecyldimethylammonium bromide, which are part of the developed product, is significantly lower than the minimum inhibitory concentration of silver ions, silver nanoparticles stabilized with citrate ion or DDAB. After analyzing the obtained experimental data, we can talk about the synergistic antibacterial effect of silver nanoparticles stabilized by didecyldimethylammonium bromide on a microbial cell.

At the next stage of the studies, the fungicidal activity of nanosilver in relation to the *Penicillium roqueforti* fungus was determined. The results of the determination of the fungicidal activity of the nanosilver preparation with different concentrations (1 - 0.5 mg/mL Ag, 2 - 0.05 mg/mL Ag, 3 - 0.005 mg/mL Ag, 4 - 0.0005 mg/mL Ag) in relation to *Penicillium roqueforti* are shown in Figure 2.

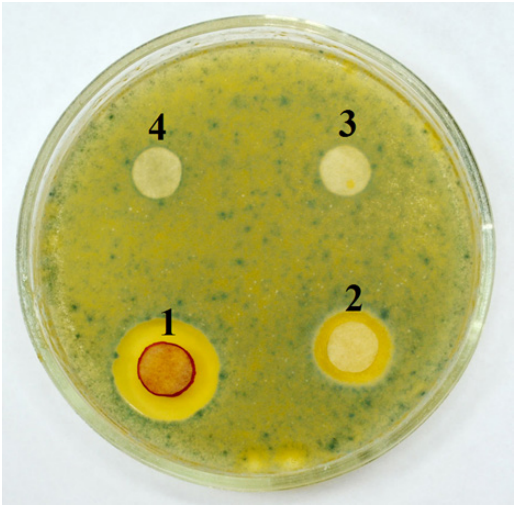


Figure 2. Photo of a Petri dish with a *Penicillium roqueforti* mold culture with discs impregnated by nanosilver samples with different concentrations (1 - 0.5 mg/mL Ag, 2 - 0.05 mg/mL Ag, 3 - 0.005 mg/mL Ag, 4 - 0.0005 mg/mL Ag)

Figure 3. Photo of a Petri dish with mold culture *Penicillium roqueforti* with discs impregnated by nanosilver samples of stabilized with DDAB, with different values of the active acidity of the medium (pH): 1. pH = 3; 2. pH = 5; 3. pH = 6; 4. pH = 8; 5. pH = 9; 6. pH = 11

As an analysis of the data showed, samples with nanosilver concentrations, C (Ag) = 0.5 mg/mL, 0.05 mg/mL, significantly inhibit the vital activity of *Penicillium roqueforti* culture. At concentrations of C (Ag) = 0.005 mg/mL, 0.0005 mg/mL the developed preparation does not possesses fungicidal activity.

Then, the fungicidal activity of the nanosilver preparation at various pH values was studied with respect to the *Penicillium roqueforti* mold culture (Figure 3).

As the analysis of Figure 3 showed all 6 test samples suppress the vital activity of the mold culture of *Penicillium roqueforti*. For greater clarity, the dependence of the suppression zones diameter on the active acidity of the medium is shown in Figure 4.

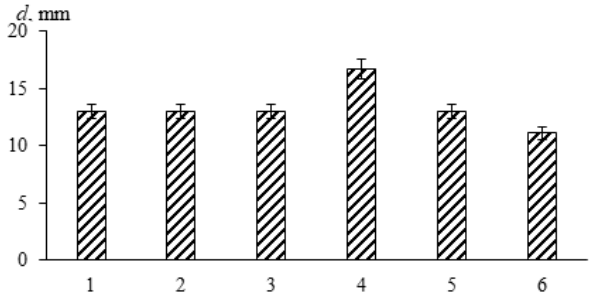


Figure 4. The dependence of the diameter of the growth inhibition zones of the *Penicillium roqueforti* fungus on the active acidity of the medium:
1. pH = 3; 2. pH = 5; 3. pH = 6; 4. pH = 8; 5. pH = 9; 6. pH = 11

As the analysis of Figure 4 showed, the largest growth inhibition zone of the mold culture of *Penicillium roqueforti* (d = 16.7 mm) is possessed by the fourth sample of colloidal silver preparation (with pH = 8). The 6th sample of colloidal silver preparation (pH = 11), has the smallest zone of *Penicillium roqueforti* growth inhibition (d = 11.1 mm), all of the other samples showed approximately the same level of fungicidal activity (diameter of the suppression zone 13 ± 0.5 mm).

4. Conclusions

- As a result of the studies, it was found that nanosilver stabilized by didecyldimethylammonium bromide exhibits high bactericidal and fungicidal activity against *Escherichia coli* and *Penicillium roqueforti*, respectively.

- It is important to note that this nanosilver preparation exhibits these properties in a wide range of pH, and thus can be used as a disinfectant base in alkaline and acid detergents and disinfectants, actively inhibiting the vital activity of fungal and bacterial cultures.

Acknowledgement

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5. References

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