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SUSCEPTIBILITY OF ESCHERICHIA COLI TO GREEN SYNTHESIS SILVER NANOPARTICLES OBTAINING ON VEGETAL EXTRACTS

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Abstract. *Resistance of E. coli to nanodispersed systems, obtained on the basis of vegetal extracts and silver nitrate, has been determined in liquid nutrient medium. Vegetal extracts got by water maceration of berry pomaces with ultrasound treatment. Zeta potential of obtained AgNPs were in a band from -15 to -24 mV and average sizes of particles were 80-92 nm. Antimicrobial action of silver nanosystems was tested in 10-times reiterative; the statistical processing of results involved using S-criterion for rejecting gross errors and calculating the average value of suspension optical density with mean square deviation. Control absence of growth was performed on Endo agar. Silver nanosystems on grape crests and pomace, and currant pomace extracts were stable and active against E. coli UKM B-906. The minimal bactericidal concentration is 7,5% for the silver nanosystem on grape extract, and 10% for currants extract. Minimal inhibitory concentration are 3,5% and 4,2% correspondently.*

Keywords: *silver nanoparticles, berry pomace extract, E. coli, minimal bactericidal concentration, minimal inhibitory concentration*

Introduction

Synthesis of silver nanoparticles (AgNPs) has the high interest to the scientific community because of their broad application in the cosmetic, therapeutic and pharmaceutical industry. Silver in various forms including silver metal, silver nitrate, silver sulfonamide, silver acetate, and silver protein are well known for their antimicrobial properties [1]. Silver based compounds are demonstrated strong biocide effects on Gram-positive and Gram-negative bacteria, mold fungi, viruses and have been used as in biomedical devices, textile industries, water treatment, and food packaging.

Nowadays the great research attention takes place to nanosized materials and metal nanoparticles. Nanosized silver particles with designed surface and structural properties perform an important material with numerous physical (catalytic, electronic, and optical), biological and pharmaceutical applications. In medicine they currently put the great impact in diagnostics, drug delivery, gene therapy, and tissue engineering [2].

The antimicrobial activity of silver nanoparticles (AgNPs) very strongly depends on size of nanoparticles, which play an important role in the inactivation of the microorganisms. Nanoparticles of size range less than 100 nm have become an area of extensive research due to their antimicrobial properties attributed to their large surface area [3].

Synthesis of AgNPs is generally carried out by various physical and chemical methods [4-7]. Most of them are successful, but have some limitations in implementation due to expensive chemical reagents and hazard to environment. In contrast, «green» nanotechnology integrates the principles of chemistry and engineering for producing eco-friendly, safe nanoparticles don't using toxic chemicals in their synthesis protocol and various waste materials have also been utilized [8]. The use of plant extracts, enzymes, bacteria, fungi, and algae provides an environmentally safe route for the production of nanoparticles. The main focus of nanotechnology is to synthesize monodispersed nanoparticles with predictable shape, size, and polymeric coating for potential biomedical applications [2].

The antimicrobial activity of silver nanoparticles against *E. coli* has been investigated by some researches [5,7,9]. Silver nanoparticles was obtained by reaction between silver nitrate and ascorbic acid in the presence the sodium salt of a high-molecular-weight naphthalene sulfonate formaldehyde condensate as stabilizing agent. Getting precipitate was washed five times with deionized water and the obtained the nanosize silver powder by freeze drying. Transmission electronic microscopy demonstrated the average size of obtained silver nanoparticles which reach to 5-20 nm. Finally, powder was dissolved in water and estimated the bactericidal effect in concentration 1-10 % in solid and liquid Luria–Bertani medium with *E. coli*. Results of experiment demonstrated that 100 % inhibition of bacterial growth in agar medium was in plates with 5 % and more concentration of silver nanoparticles powder. In contrast, in liquid medium all estimated concentration didn't have full bactericide effect, but significant grows delay of *E.coli* culture performed in tubes with 10% concentration of silver nanoparticles. Additional analysis was provided due to scanning and transmission electron microscopy for study the biocidal action. Thus, *E. coli* cells were damaged, showing formation of "pits" in the bacteria cell wall [9].

The aim of present research is *in vitro* estimation of bactericidal effect for first-time received original "green synthesis" system of AgNPs, obtained by reducing $AgNO_3$ due to compounds of aqueous vegetal extracts, on *Escherichia coli.*

Materials and methods

Material

Strain Escherichia coli UKM B-906 was received from Danylo Zabolotny

Institute of Microbiology and Virology National Academy of Science of Ukraine. Bacteria were cultivated in liquid medium meat-peptone broth (MPB) during 6 hours in thermostat with 370C. Before conducted bactericidal tests, inoculum suspension was diluted with medium up to optical density 0.5 of McFarland standard scale that corresponded 108 colony-forming units CFU per cm^3.

Obtaining of AgNPs and characterization techniques

In first, the vegetal extracts were obtaining due to water maceration of berry pomaces with ultrasound (US) treatment. As a plant material for extraction we used such food wastes as crests and pomace of grape, pomegranate pomace, and currant pomace. Sonication was performed for 1 h with frequency 27 kHz and power 200 W. Obtained extract after cooled down was passed through Whatman filter (# 1) for getting homogeneous aqueous disperse system.

In second step, the dispersions of silver nanoparticles were prepared by chemical reaction using vegetal extract as the natural reducing for argentum nitrate and stabilizing of dispersion system agent. Equal volumes of aqueous plant extract were dropwise added to 0.01 M AgNO3 water solution. The color of the reaction mixture gradually changed over 30 min and as well indicated the formation of AgNPs.

UV-Vis spectra of colloidal solutions were recorded in the wavelength range of 190-700 nm using the UV-5800 PC spectrophotometer (FRU, China) and quartz cuvettes. Zeta potential of colloidal particles was measured on a Zetasizer Nano-25 analyzer (Malvern Instruments Ltd., Malvern, England).

Bactericidal tests

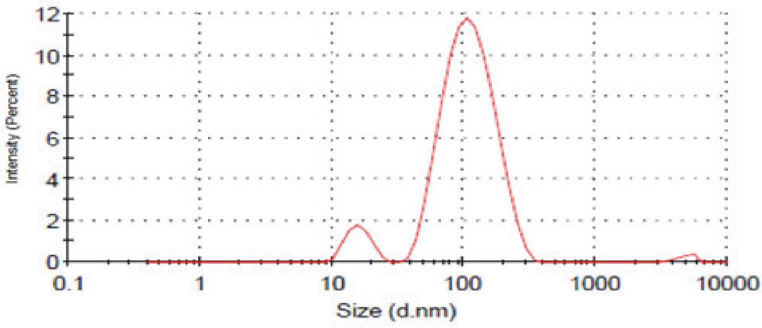
Bacterial susceptibility measured quantitatively in a particular test system and expressed as the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the agents for the strain tested [10]. Active concentrations estimation in liquid medium was performed by the method of evaluation of the action of antibiotics [11], and we used MPB as non-selective nutrient medium. In set of tubes that covered 2 cm^3 of MPB we added 2 cm^3 of AgNPs solution with concentration range and finally added 2 cm^3 of *E. coli* inoculum. In control tube we put 2 cm^3 of MPB, 2 cm^3 of aseptic distilled water, and 2 cm^3 of inoculum. Experiment carried out in 10-times reiterative for ensure of result reproducibility. All tubes incubated in thermostat with 37^0C in 24 hours.

After incubation period we conducted photometrically monitoring turbidity (colorimetric changes) in the broth in which the test organism is growing. The results was evaluated as difference in optical density of the suspensions by photoelectric colorimeter with 5 mm quartz cuvette at λ = 540 nm (for most samples) or 670 nm and 750 nm (for dark opaque samples with high concentration of silver nanoparticles solution). As additional control was carried out taking samples from each tube and put on solid selective medium Endo agar in Petri dishes, which were incubated for at 370C in two days and, finally, analyzed visually for present or absent bacteria growth.

Results and discussion

Chemical characteristics of obtained disperse system

Formation of silver nanoparticles was monitored by UV–vis spectrum and



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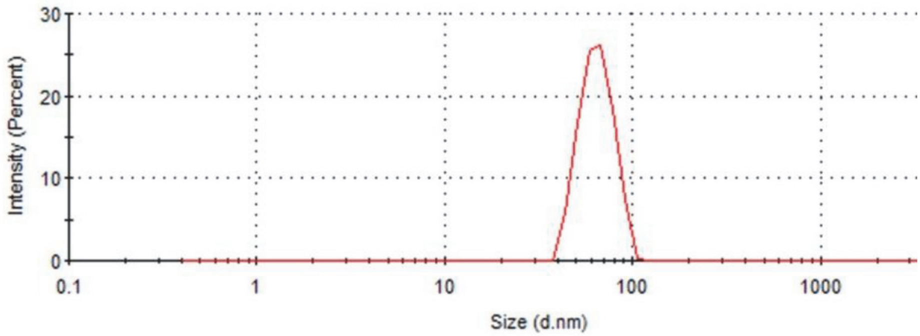

Fig. 1. The silver nanoparticle size distribution by intensity obtained by the various extracts: a - grape pomace, b – currant

concluded that reaction was completed within 30 min of exposure. Dynamic light scattering (DLS) used for measure the hydrodynamic size of nanoparticles in suspensions. The chart with sizes distribution frequencies of two hours after synthesized AgNPs is showed on Fig.1.

Zeta potential measurements were conducted to evaluate the stability of the disperse system (table 1). In general, a suspension that exhibits a zeta potential more than –20 mV (less than the absolute value of a number 20) is considered unstable and will result in particles settling out of solution in the absence of other factors.

Antibacterial properties

MBC was described as the lowest concentration of AgNPs solution in tube in which there wasn't significant changes of turbidity and found any colony in Endo agar. MIC is estimating as concentration in tube in which turbidity (optical density) was doubly decreased.

1. Characteristics of silver nanoparticles synthesized using the various extract

NP	Zeta potential, ΔmV	Average NP size, nm
AgNPs on grape extract	-15.31	92
AgNPs on currant extract	-24.23	80

2. Optical density of test tubes content set and growth on Endo agar

AgNPs on grape extract						
	Control	1%	5%	7,5%	10%	15%
Opt.den	0,52± 0,015	0,43± 0,008	0,12± 0,016	0,03± 0,005	0,024± 0,005	0,025± 0,005
Growth on Endo agar	+	+	+	–	–	–
AgNPs on currant extract						
	Control		5%	7,5%	10%	
Opt.den	0,54± 0,02		0,17± 0,015	0,13± 0,005	0,075± 0,004	
Growth on Endo agar	+		+	+	–	

Experimental results of susceptibility of *E. coli* strain cultivated in liquid nutrient medium with addition concentration range of silver nanoparticles solutions, obtained on a base of different vegetal extract are present in table 2. Statistical processing of experiment results were as rejection of gross errors using the S-criteria and the calculation of the average value of optical density with mean square deviation.

Low values of optical density at least less than 0,075 as well absent of

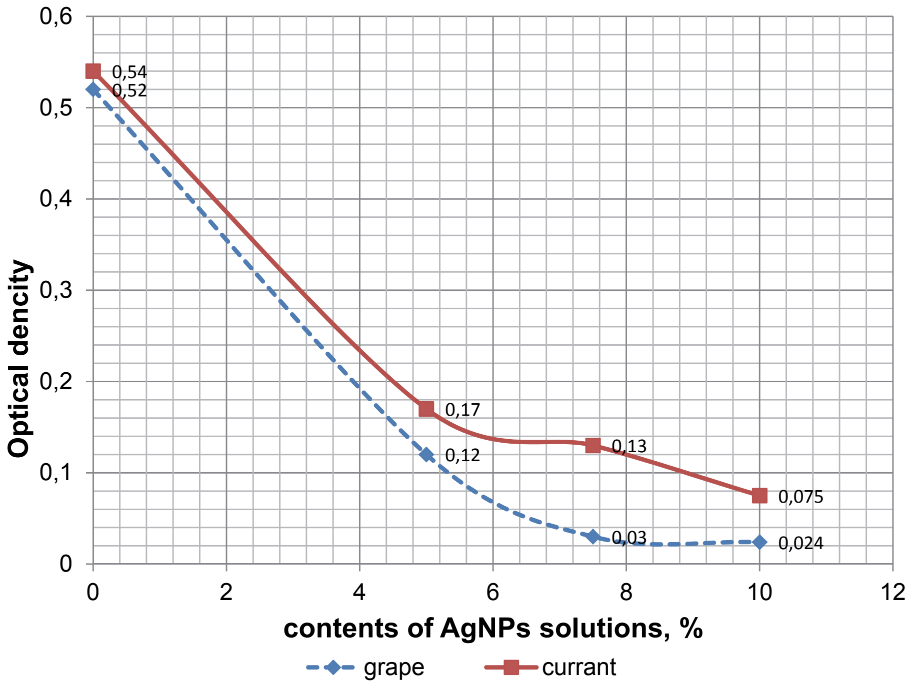

Fig. 2. Chart for graphical extrapolation and estimation of MIC of AgNPs obtained on a base vegetal extracts

bacteria growth on Endo agar allow us to determinate the minimal bactericidal concentration of each sample of AgNPs solutions.

For estimation and compare the minimal inhibitory concentrations of different AgNPs solutions have been used graphical method as determination of agent concentration that are corresponded to half value of optical density in relation of control tube in each sample (fig 2.)

Therefore, for Ag NPs obtaining on a base of grape extract MBC is 7,5 % and MIC is 3,5%; for currant extract MBC is 10 % and MIC is 4,2 %.

Conclusion

As experiment demonstrated, the most effective system against *E. coli* UKM B-906 was a disperse system of silver nanoparticles obtained from $AgNO_3$ and grape crests and pomace extract which contained the optimal ratio of compounds as excellent reducing of argentum salt and stabilizing of nanodisperse system agents. Probably, the active molecules present in the extracts such as polyphenols and triterpenes promote the reduction of metal ions.

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Анотація. *Була визначена активність нанодисперсних систем, одержаних на основі рослинних екстрактів та нітрату срібла щодо E. coli в рідкому середовищі. Рослинні екстракти були отримані з ягідних вичавок методом водної мацерації з обробкою ультразвуком. Дзета-потенціал утворених наночасток срібла був в межах від -15 до -24 мВ, а самі наночастки мали розмір 80-92 нм. Дослід з випробовування антимікробних властивостей наносистем срібла закладали в десятикратній повторності, статистична обробка результатів передбачала використання S-критерію для відкидання грубих похибок та розрахунок середнього значення оптичної густини суспензії з урахуванням середнього квадратичного відхилення. Контрольні висіви проводили на Ендо агар. Наносистеми срібла, одержані на основі екстракту гребенів і вичавок винограду та вичавок смородини виявились стабільними та активними відносно E. coli UKM B-906. Мінімальна бактерицидна концентрація становила 7,5 % для наносистем срібла з екстракту винограду та 10 % – для екстракту смородини. Мінімальні інгібуючі концентрації становили відповідно 3,5 % та 4,2 %.*

Ключові слова: *silver nanoparticles, berry pomace extract, E. coli, minimal bactericidal concentration, minimal inhibitory concentration*