BIOGENIC SILVER NANOPARTICLES

Zaynitdinova L.I., Juraeva R.N., Kukanova S.I., Vokhidova N.R., Tashpulatov J.J., Lobanova I.V.

Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan

e-mail: zajn-lyudmila@yandex.ru

Abstract: Capability of different microorganisms to produce nanoparticles of silver (NPAg) at different pH levels was studied. It was established that pH of the reaction medium considerably affects form and size of produced nanoparticles. The fact of NPAg formation produced by the different microorganisms was studied by UV-spectroscopy and AF-microscopy methods.It was determined that in the selected conditions of the synthesis both clusters and nanoparticles of silver are produced ranging in size from 20 to 300 nm and form absorption bands at λ_{max}275 nm and 420 nm, respectively. It was revealed that changeof pH level of the reaction system may result in regulation of the size and form of the nanoparticles.

Key words: microorganisms, cultural broth, biosynthesis of silver nanoparticles

INTRODUCTION

Due to unique features nanoparticles attract considerable attention now. Many characteristics of materials at the nanoscale greatly differ from the features of the same materials at the larger scales. Though nanosized materials may be produced with use of different traditional physical and chemical methods, nowadays it is possible to synthesize nanomaterials biologically with application of environment friendly methods. Recently the merge of nanotechnologies and biology resulted in establishment of the new field – nanobiotechnology, which includes application of biological objects, such as viruses, bacteria, fungi, yeasts, algae and plants, in a number of biochemical and biophysical processes [1].Microbiological reduction of metals may be a reasonable strategy for re-use of metal-containing pollutants and wastes. Bacteria capable to mobilization and immobilization of metals, and in some cases bacteria capable to restore ions of metals, exhibit the ability to sediment metals at the nanoscale. Biosynthesis of nanoparticles (NP)with application of bacteria becomes fast developing field [2].Optimization of the processes may result in sythesis of NP with necessary morphology and controlled sizes [3].Biological systems, especially bacteria, due to their fast growth velocity, are unique candidates for achieving this goal[4,5].

There are many reports availableon microbial synthesis of nanoparticles. Extracellular synthesis of 16-40 nm NPAg by *Pseudomonas strutzeri*was studied [6,7]. It was established that microbial synthesis of NP of metals may take place both intra- and extra-cellularly [8,9].Extracellular synthesis of NP is often observed, moreover it is considerably cheaper.These features contribute to large scale production of the NPAg and increase possibilities for potential application of nanoparticles in practice. Diversityof microorganisms participating in such processes results in multiplicity of such studies [10]. Thus, the ability of bacteria from *Bacillus*genus to form NPAg was established [11].Other researchers study filamentous fungi as potential agents for production of NP. Ahmad et al. established extracellular synthesis of nanoparticles of CdS by *Fusarium oxysporum* [12]. Khan et al. conducted a very convincing study on optimization of such parameters as pH level, concentration of the $AgNO_3$ and fungal biomass to receive NPAg of the certain shape and size[13]. Nevertheless, it necessary to point out that many researchers focused on determination of the pH level impact by the method of chemical restoration. The absorption spectra of all tested solutions revealed the peak of surface plasmon at the wavelength ca. 420 nm [14]. Higher level of pH resulted in receipt of NPAg of the smaller size compared to such received at the lower pH level. This difference may be explained by difference in velocity of restyoration of the precursor. In addition to the inverse proportionality between size and pH level, it is obvious that increase in pH level allows to receive spherical NP, wheras the lower pH level results in formation of rods and particles of triangular shape [14].

MATERIALS AND METHODS

To receive NPAg the standard solutions of $AgNO_3$of different concentration (from 25 to 100 Ag^+ mg/l) were prepared. Solution of $AgNO_3$ were added to the cultural broth. Mixture of cells and ions of silver was incubated on rotary shaker at 180 rpm and 28°C for 2-3 days. Formation of the NPAg was observed visually by staining of solutions in characteristic colors.

Cultivation of microorgainisms was condcuted on meet extract peptone broth (MEPB) diluted two times.

Strain *Pseudomonas stutzeri*used in this study was isolated from a mixed population of microorganisms inhabiting soils polluted with different xenobiotics. The choice of the object was determined by its resistance towards different pollutants including heavy metals and by ability to biosorption of silverб since it is presumed that ability to produce nanoparticles of metals is defensive function of the microorganisms [15].

Antimicrobial activity was determined by standard methods [16].

UV-spectroscopy was conducted on spectrophotometer Specord 210(Germany) within range 190-1000 nm. Accuracy of UV photometry with potassium dichromate is in accordance with Ph.Eur.±0.01.

Optic study was conducted with use of the optical microscope Leica 1000 (Germany) with magnification rate ×40 - ×1300.

Morphology of films of nanostructured systems was studied with use of atomic force microscope Agilent 5500 (USA) at the room temperature. Silicon cantilevers with hardness 9.5 N/m with frequency 145 kHz were used. Maximum field of scanning on AFM by X,Y was 15×15 µm2, by Z – 1 µm.

RESULTS AND DISCUSSION

Different representatives of microbial community were initially analysed [17,18]. It is necessary to note that representatives of *Pseudomonas*genus along with representatives of *Bacillus*genus support stucture of microbial communities at the sress situations and at the same time they may be active producers of NPAg [19,20].A microbial strain was isolated from a mixed population of microorganisms inhabiting soils polluted with different xenobioticsand was used in this studybased on preliminary results. Experiments on impact of the initial pH level of the medium on biosynthesis of NPAg by *Pseudomonas stutzeri*was conducted (table 1).

Table 1: Impact of the pH level on formation of NPAg by *Pseudomonas stutzeri*(initial color of the cultural broth – light-biege; incubation:72-120 h)

#	Ag^+concentration, mg/l	pH	Color
1	75	5.0	Light-beige muddy
2	75	6.0	Brown muddy
3	75	7.0	Beige muddy (72 h)

4	75	8.0	Brown muddy
5	75	8.5	Dark brown muddy
6	75	9.0	Dark brown muddy
7	75	7.0	Black muddy (120 h)

Impact of adding $Ag^+$was assessed both by the visual change of cultural broth and by the weight of produced biomass. It is known that change of color of the microbial broth at addition of solutions of silver is a marker for formation of NPAg. Conducted test revealed that at pH 5.0 the initial transparent light-biege color after incubation has changed to light-biege muddy, whereas at pH level from 6.0 to 9.0 the broth color has changed from light-biege to yellow and then to dark brown(picture 1).

a

b

Picture 1. Impact of pH level (5.0; 6.0; 8.0; 8.5; 9.0) on development of *Pseudomonas stutzeri* and biosynthesis of NPAg: a – initial culture, b – after formation of NPAg.

UV-spectra were registered within 190-600 nm, which cover plasmon resonance spectra of silver NP, clusters and electronic transitions of its ions. It is obvious, that as in case of chemical reduction, the absorption bands ofall tested samples revealed peak of surface plasmon at the wave length λ_{max} 420 nm and at 250-280 nm, corresponding with presence in the system of both NP and clusters of silver, respectively.

UV-spectroscopy revealed that with increased pH level of the medium, respectively, the intensity of absorption bands increases as well, thus the quantity of NPAg. The visual observation of the reaction mixture testifies this fact, since color intensity of the solution in alkali media reasonably faster changes from light-biege to dark brown and black (sample #7) compared to acid media (picture 2).

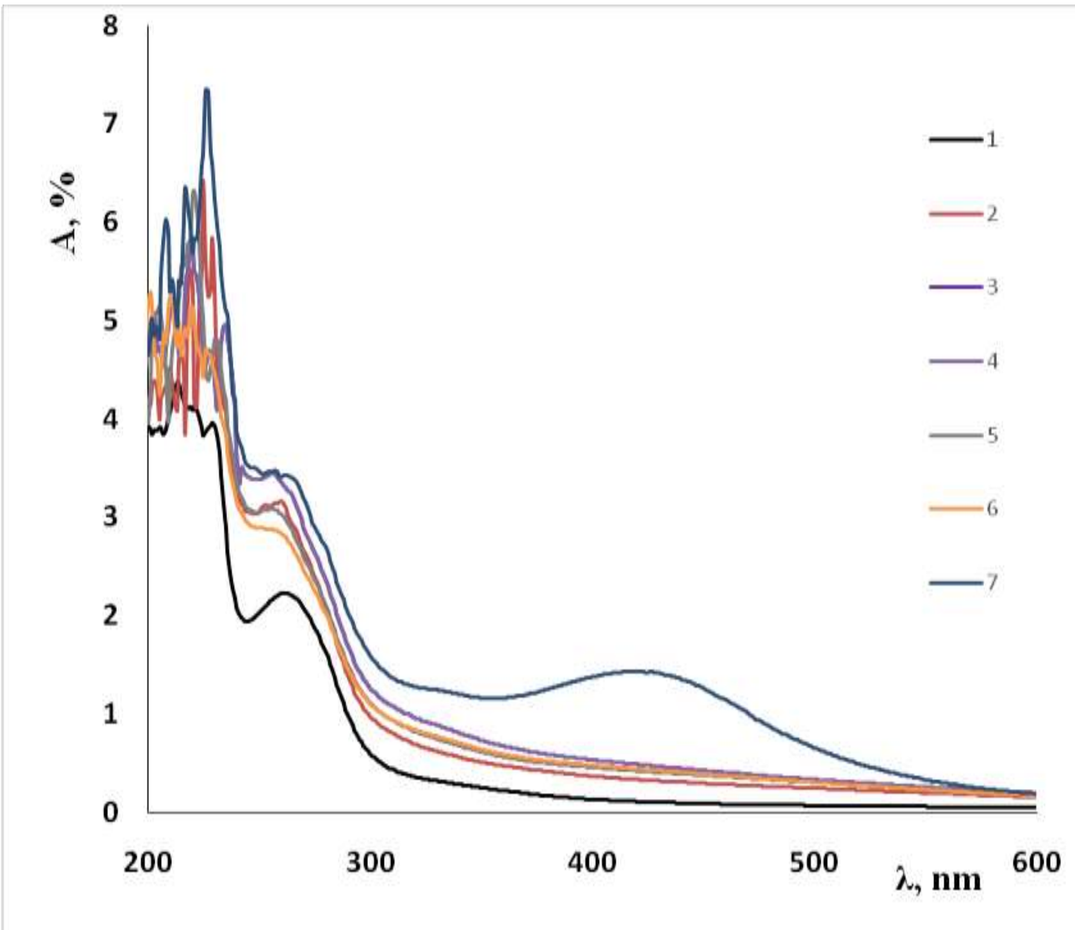
Analysis of spectra reveals that in light acid and neutral media (pH 6.0 and 7.0) the process of NP formation runs slowly and mainly clusters of silver are formed at 275 nm, whereas with higher pH the intensity of absorption bands increases and they move to long-wave region λ, which may be linked with size and shape of NP.

It is necessary to note that increase of pH level leads to increased process of reduction of silver ions, which, respectively, leads to aggregation of particles. In these regards, at pH 7.0(Ag^+75mg/l; sample # 7) particles are larger in size and form absorption bands at λ_{max} 425 nm.

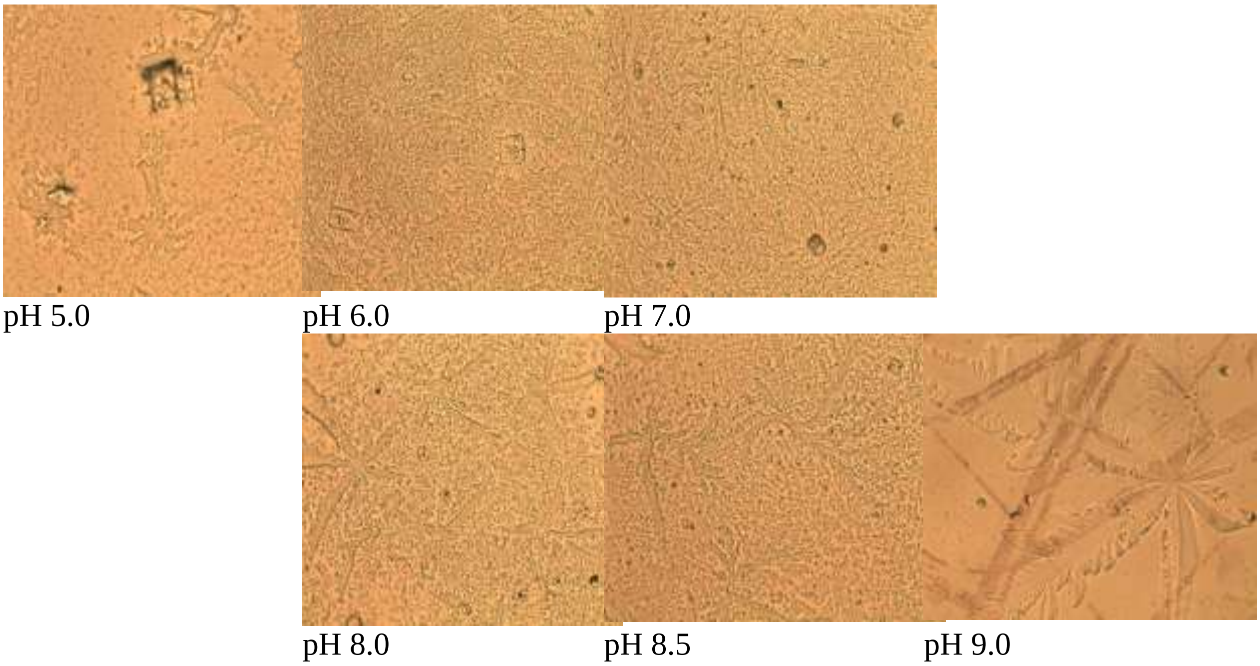
Microscopy study confirm that pH of the medium of the reaction system (samples ## 1-6) and concentration of silver ions (sample #7)affect the size and shape of produced NP (picture 3).

It was established that in alkali medium (pH 8.0 – 9.0) the dendrite structure is observed, while in light acid (pH 5.0 – 6.0) crystals of cubic and cylindrical form are produced.

The neutral medium (pH 7.0) results in formation of both spherical and non-spherical (dendritic)NPAg, increase of Ag^+ concentration from 75 mg/l to 100 mg/l results in formation of NP of cubic shape.



Picture 2. UV-spectra of samples of NPAg formed in MEPB with different pH levels (1-7 samples)



Picture 3.Microscopy images of NPAg formed at the different pH levels of the medium (Ag^+ 75 mg/l).

Fast formation of the resistance of microorganisms to antibiotics demands necessity for search of the new alternative antimicrobial preparations [21, 22].Antibacterial activity of nanoparticles attracts extensive attention due to their high physical and chemical stability.NPpossess attractive antibacterial activity due to their enlarged specific surface, which leads to the increased reactivity of the surface. When NP are suspended in the biological culture they interact with different biological interfaces resulting from presence of such cellular particles as DNA, protein, lipids, polysaccharides and so on, which in many cases promotes to antibacterial characteristics. NP help to control not only malignant bacteria, but also microscopic fungi.In these regards, metals in the form of NP are one of the perspective pretenders for establishment of the new class of antibacterial means, since they possess low toxicity, prolonged action; their biotic doses stimulate functional activity of enzymatic systems[23].

Many studies are focused on possibility of use of NP in medicine [24,25].Bactericidal activity of NPAg against different individual strains of microorganisms is studied well enough. From practical point of view it is necessary to study antimicrobial features of the water dispersions of silver and other metals, since interaction of nanomaterials with microflora is characterized by a number of unusual physical and chemical and biological properties, their toxicological characteristsics change as well.

Nowadays, besides of using chemotherapeutic agents,the antimicrobial therapy is widely applied to combat pathogens of purulent-inflammatory disease.That is why the study of antimicrobial activity of subinhibiting concentrations of water dispersions of NPAg represents certain interest. It is necessary to evaluate perspectives of their utilization as highly active means for the treatment of purulent inflammation of the skin and soft tissues.

In these regards, the antimicrobial analysis of microbiologically produced NPAg was conducted. The antimicrobial activity both of cultural broth containing NPAg and cell-free suspension with NPAg was studied. To receive cell-free suspension the cells from the reaction solution were removed by centrifugation at 6000 rpm for 20 min, and then cell-free fraction was analyzed for antimicrobial activity. It was established that the cultural broth containing NPAg and cell-free suspension with NPAg, at concentration of Ag^+75 and 100 mg/l,showed the antibiotic activity against all test cultures (the zone of pathogen's growth suppression was 15-32mm), whereas lower concentration of NPAgwas active towrds *Candida albicans*(table 2).

Table 2: Impact ofbiogenic NPAgon growth and development of test cultures

#	Initial concentration of $AgNO_3$	Antagonistic activity (diameter of the zone of growth suppression), mm				
		Bacillus subtilis	***Escherichia coli***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Candida albicans***
1.	25 mg/l	-	-	-	-	18.0
2.	50 mg/l	-	-	-	-	22.0
3.	75 mg/l	18.0	18.0	22.0	22.0	30.0
4.	100 mg/l	22.0	17.0	22.0	22.0	17.0
5.	25 mg/l	-	-	-	-	20.0
6.	50 mg/l	-	-	-	-	12.0
7.	75 mg/l	18.0	18.0	20.0	20.0	32.0
8.	100 mg/l	15.0	17.0	22.0	15.0	20.0

**1-4 – culturalbrothcontainingNPAg;*
*** 5-8 – cell-free suspension containing NPAg.*

Obtained results and analysis of available scientific reports reveals efficiency of nanoparticles as antibacterial agents. Moreover, application of silver in the form of NP provides an opportunity to decrease considerably its concentration compared to the ion form with preservation of its antimicrobial features. NPAg are less toxic than the ion form, which expands the possible spectrum of their application in the medicine. Of special importance are both the development of non-toxic antimicrobial preparations on basis of NPAg, preserving their bactericide features and stability during long term, and the study of mechanisms of their antimicrobial action.

CONCLUSION

ThepossibilityforrestorationofsilverionsinmicrobialculturalbrothwithdifferentpHleveloftheme diumwasestablished. The change in color of the solution from light-yellow to dark brown was visually observed, which is indirect confirmation that in selected conditions silver ions are restored and metal nanoparticles are formed.

Conducted study on pH sensitivity of NP-synthesizing microorganisms is stipulated by possible application of pH-sensitive NP for transportation of different substances within organism. Different organs, tissues and subcellular components, and their pathophysiological conditions as well, may be characterized by different pH levels. Our study may lead to reconsideration of the current opinion that NP are only passive means of delivery towards role of the sensitive means, which represent different adaptive features of microorganisms in media different by pH level.

Ability of microorganisms to produce extracellular NPAg at the different pH levels was studied. It was established that pH level of the medium considerably affects the shape and the size of produced NP. At addition of silver ions to the cultural broth of bacteria the different NP ranging from 20nm to 300 nm are produced, which may further form agglomerates.The formation of NP at the presence of microorganisms was studied with use of UV-spectroscopy and AF-microscopy methods. Results reveal that UV-spectra of studied samples contain absorption bands corresponding to both clusters and NP of silver at λ_{max}275 nmandλ_{max}420 nm, respectively.

Literature cited

[1] Marooufpour, N., Alizadeh, M., Hatami, M., &Lajayer, B. A. (2019). Biological Synthesis of Nanoparticles by Different Groups of Bacteria. In Microbial Nanobionics (pp. 63-85). Springer, Cham.

[2] Yusof, H. M., Mohamad, R., &Zaidan, U. H. (2019). Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review.Journal of animal science and biotechnology,10(1), 57.

[3] Iravani, S. (2014). Xenorhabdusstockiae KT835471-mediated feasible biosynthesis of metal nanoparticles for their antibacterial and cytotoxic activities.Int Sch Res Notices,2014, 1-18.

[4] Ghashghaei, S., &Emtiazi, G. (2015). The methods of nanoparticle synthesis using bacteria as biological nanofactories, their mechanisms and major applications.Current Bionanotechnology,1(1), 3-17.

[5] Arshad, A. (2017). Bacterial synthesis and applications of nanoparticles. Nano Sci Nano Technol, 11(2), 119.

[6] Klaus-Joerger, T., Joerger, R., Olsson, E., & Granqvist, C. G. (2001). Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. TRENDS in Biotechnology, 19(1), 15-20.

[7] Mann, S. (2001).Biomineralization: principles and concepts in bioinorganic materials chemistry(Vol. 5). Oxford University Press on Demand.

[8] Waghmare, S. S., Deshmukh, A. M., & Sadowski, Z. (2014). Biosynthesis, optimization, purification and characterization of gold nanoparticles.African Journal of Microbiology Research,8(2), 138-146.

[9] Kalishwaralal, K., Deepak, V., Pandian, S. R. K., Kottaisamy, M., BarathManiKanth, S., Kartikeyan, B., &Gurunathan, S. (2010). Biosynthesis of silver and gold nanoparticles using Brevibacteriumcasei. Colloids and surfaces B: Biointerfaces, 77(2), 257-262.

[10] Varshney, R., Bhadauria, S., & Gaur, M. S. (2012). A review: biological synthesis of silver and copper nanoparticles. Nano Biomedicine & Engineering, 4(2).

[11] Saravanan, M., Vemu, A. K., & Barik, S. K. (2011). Rapid biosynthesis of silver nanoparticles from Bacillus megaterium (NCIM 2326) and their antibacterial activity on multi drug resistant clinical pathogens. Colloids and Surfaces B: Biointerfaces, 88(1), 325-331.

[12] Ahmad, A., Mukherjee, P., Mandal, D., Senapati, S., Khan, M. I., Kumar, R., & Sastry, M. (2002). Enzyme mediated extracellular synthesis of CdS nanoparticles by the fungus, Fusarium oxysporum. Journal of the American Chemical Society, 124(41), 12108-12109.

[13] Khan, N. T., & Jameel, J. (2016). Optimization of reaction parameters for silver nanoparticles synthesis from Fusarium oxysporum and determination of silver nanoparticles concentration. J Material Sci Eng, 5(283), 2169-0022.

[14] Alqadi, M. K., Noqtah, O. A., Alzoubi, F. Y., Alzouby, J., &Aljarrah, K. (2014). pH effect on the aggregation of silver nanoparticles synthesized by chemical reduction. Materials Science-Poland, 32(1), 107-111.
[15] Khandel, P. & Shahi, S.K. (2016). Microbes mediated synthesis of metal nanoparticles: current status and future prospects. Int J NanomaterBiostruct, 6(1),1-24.
[16] Yegorov, N.S. (1965). Microbes antagonists and biological methods of determination of antibiotic activity. Moscow, p. 131.
[17] Zaynitdinova L.I., Kukanova S.I., Juraeva R.N., Ergashev R.B.,&Tashpulatov J.J. (2020). Synthesis of copper nanoparticles in presence of microorganisms. Journal of critical reviews, 7(11), 1641-1647.
[18] Zaynitdinova L., Vokhidova N., Rashidova S., Kukanova S., Juraeva R., &Tashpulatov J. (2018). Microbial synthesis of silver nanoparticles by Pseudomonas sp. Biotechnology: An Indian journal, 14(4), 0-9.
[19] Singh, H., Du, J., Singh, P., & Yi, T. H. (2018). Extracellular synthesis of silver nanoparticles by Pseudomonas sp. THG-LS1. 4 and their antimicrobial application. Journal of pharmaceutical analysis, 8(4), 258-264.
[20] Kumar, P., Mahajan, P., Kaur, R., & Gautam, S. (2020). Nanotechnology and its challenges in the food sector: a review. Materials Today Chemistry, 17, 100332.
[21] Khandel, P., Shahi, S. K., Soni, D. K., Yadaw, R. K., & Kanwar, L. (2018). Alpinia calcarata: potential source for the fabrication of bioactive silver nanoparticles. Nano convergence, 5(1), 37.
[22] Hajipour, M. J., Fromm, K. M., Ashkarran, A. A., de Aberasturi, D. J., de Larramendi, I. R., Rojo, T., ... &Mahmoudi, M. (2012). Antibacterial properties of nanoparticles. Trends in biotechnology, 30(10), 499-511.
[23] Patent № 2446810 (Russian Federation). Gluschenko, N. N., Bogoslovskaya, O. A., Rakhmetova, A. A., & Alekseeva, T. P. (2012).Antimicrobialagents.
[24] Usha, R., Prabu, E., Palaniswamy, M., Venil, C. K., & Rajendran, R. (2010). Synthesis of metal oxide nano particles by Streptomyces sp. for development of antimicrobial textiles. Global J BiotechnolBiochem, 5(3), 153-160.
[25] Singh, R., Shedbalkar, U. U., Wadhwani, S. A., &Chopade, B. A. (2015). Bacteriagenic silver nanoparticles: synthesis, mechanism, and applications. Applied microbiology and biotechnology, 99(11), 4579-4593.