Chapter

Silver Nanoparticles Offer Effective Control of Pathogenic Bacteria in a Wide Range of Food Products

Graciela Dolores Avila-Quezada
and Gerardo Pavel Espino-Solis

Abstract

According to the Food and Agriculture Organization (FAO), food wastage still causes massive economic loss. A major role in this loss is played by the activities of microbial organisms. Treatments such as heat and irradiation can reduce microorganisms in fruits and vegetables and hence reduce postharvest loss. However, some of these treatments can injure the fruit. Effective chemical treatments against bacterial infestations can result in resistance. A more recent method is the use of silver nanoparticles. These can act in a number of ways including at cellular level by inhibiting the cell wall synthesis, by binding to the surface of the cell membrane and by interposing between the DNA base pairs, and by inhibiting biofilm formation, affecting the thiol group of enzymes, affecting bacterial peptides and hence interfering with cell signaling and attaching to the 30S ribosome subunit. A ground-breaking way to survey the effects of the silver nanoparticles on bacterial populations is by flow cytometry. It allows measurement of many characteristics of single cells, including their functional characteristics such as viability and cell cycle. Bacterial viability assays are used with great efficiency to evaluate antibacterial activity by evaluating the physical rupture of the membrane of the bacteria.

Keywords: prevention of postharvest food losses, FAO, fruit pathogens

1. Introduction

1.1 Postharvest pathogens of fruit

Postharvest spoilage of fruits can be caused by a large number of bacterial species. Some of the most important are *Enterobacter cloacae, Erwinia herbicola*, *Lelliottia amnigena, Pantoea ananatis, Pantoea agglomerans, Pantoea allii, Enterobacter aerogenes, Pseudomonas fluorescens* and *Streptomyces* sp. [1–6]. A wide range of fungal species is similarly involved [2, 7–9].

If adequate postharvest handling and storage practices are not employed, postharvest decays of fruit and vegetables can cause losses of 50% or more [7].

The main triggers for invasion by microorganisms are physiological changes that activate ethylene synthesis or that cause changes to the cuticle or cell walls (loosening), or declines in natural antifungal compounds or high contents of carbohydrates and other nutrients and water. These changes usually occur naturally during ripening [10–12].

Postharvest contamination of fruit by human pathogens can be another key issue in the supply chain. The most commonly reported human pathogen contaminants causing disease outbreaks are bacteria such as *Escherichia coli* (*E. coli*), *Salmonella* spp., *Mycobacterium* spp., *Brucella* spp. and *Pseudomonas aeruginosa* (*P. aeruginosa*). However, good manufacturing and handling practices can significantly reduce these contaminations [13, 14].

Because of the behavior of microbial populations, including fungi and bacteria, an initial infection may originate new infection foci that appear near the primary one, so increasing disease incidence and/or severity [15, 16]. Quality deterioration and loss of fresh fruit and vegetables during storage have an exceptionally high economic impact because by this stage high costs have been incurred in harvesting, grading, packaging, freighting and storage. All these reasons emphasize the importance of defining new practices to reduce populations of the postharvest microorganisms.

2. Silver nanoparticles for pathogen control

Silver nanoparticles (AgNPs) offer oligodynamic action which is also of low toxicity and broad spectrum [17–19]. Moreover, compared with synthetic biocides, there is also only a low chance that microbial resistance might develop. These AgNPs have been exploited against Gram-negative bacteria, such as *Acinetobacter, Escherichia, Pseudomonas, Salmonella* and *Vibrio,* and against Gram-positive bacteria including *Bacillus, Clostridium, Enterococcus, Listeria, Staphylococcus* and *Streptococcus* [20]. A number of research reports have demonstrated that their antimicrobial nature depends on the surface-capping agent and the size and shape of the nanoparticle [21, 22].

The effectiveness of AgNPs also depends on bacterial dose [23]. Silver nanoparticles affect the growth of bacteria in a dose-dependent manner. In a study conducted by Agnihotri et al. [23], concentrations of 10 and 20 μg/ml Ag (10 nm) caused reductions of ~18 and ~53% in *E. coli*, respectively. Meanwhile, AgNP concentrations at 30 and 40 μg/ml eliminated all bacterial growth.

Silver nanoparticles smaller than 100 nm, and containing between 10,000 and 15,000 silver atoms, are effective as antibacterial agents [20]. The AgNPs' antibacterial potential increases as size decreases. This effect is more pronounced for AgNPs of size <10 nm, because contact with the bacterial cell is direct [24].

Research into the antimicrobial activity of AgNPs against Gram-positive and Gram-negative bacteria shows Gram-negative bacteria are more sensitive to AgNPs than Gram-positive ones [23, 25], although their relative sensitivity cannot be explained based only on a difference in the composition of the cell membrane.

In studies using discs impregnated with AgNP in culture media with bacteria, the formation of a clear zone of inhibition around the impregnated discs is an indicator of bactericidal potential of AgNP > 15 nm [21]. Bacteria are unable to survive in this area, possibly because of the release of silver in the form of nanoparticles or of silver ions.

In addition, nanoparticle silver can be released by the mobility of small size AgNPs through the semisolid agar, whereby a zone of inhibition is observed.

In a previous study conducted by Biao et al. [21], chitosan was combined with silver nanoparticles to form composites. They found that chitosan-silver colloid has a high inhibition ratio against the prokaryotes *E. coli* and *Staphylococcus aureus* (*S. aureus*) and the eukaryote *Candida albicans* (*C. albicans*). They concluded that the chitosan-silver colloid had a broad spectrum of antimicrobial activity.

3. Some mechanisms of bactericidal action of silver nanoparticles (AgNPs)

3.1 Electrostatic attraction

A way to transport active silver cations to the bacteria can occur on the cell membrane or within the cell. When combined with protonated chitosan, the positively charged AgNPs bind well to the negatively charged bacterial membrane proteins through electrostatic attraction [23].

3.2 Alterations in the bacterial membrane

The first bacterial contact with AgNP can trigger an antibacterial mechanism by facilitating the entry of AgNPs into the bacterial cells. This is followed by an explosive release of silver ions inside the bacterial cells causing the bactericidal effect.

The nature of the AgNP, bacteria interaction and its antibacterial effect have been analyzed by a number of methods. Bacteria exposed to AgNPs show high protein leakage and morphological changes [26]. As an example, *E. coli* treated with AgNPs (~10 nm) appeared to shrink and develop an irregular shape. Micrographs show AgNPs on the cell membrane attached to the lipopolysaccharide layer of the cell wall, and a proportion of AgNPs were found inside the bacterial cell [23].

Biao et al. [21] noticed that bacterial strains have intact membranes and smooth surfaces in the absence of silver colloid, whereas after exposure to chitosan-silver colloid, the cell membrane and surface become shriveled, invaginated and disrupted. This cell membrane damage indicates the mode of action of chitosan-silver colloid. Its bactericidal effect is attributed to the release of silver cation from AgNPs and to alteration of the bacterial cell wall structure and associated physicochemical changes.

Osmoregulation of the bacterial cell can also be affected causing extrusion of intracellular material and hence cell death. The deformed or wrinkled cell wall can also cause leakage of cytoplasmic contents.

In addition, AgNPs can penetrate bacterial membranes, facilitating internalization. The rupture of perforation of the cell wall is an evidence of internalization of AgNP and of uncontrolled transport through the cytoplasm resulting in cell death [27] (**Figure 1**).

3.3 Silver nanoparticles internalization: effects on DNA

Multiple pathways of AgNP can occur after internalization. Silver atoms in nanoparticles are characterized by a high affinity with sulfur and phosphorus-containing compounds such as DNA. In this way, they readily combine with cell constituents and so destroy the cell.

Silver ions can also inhibit bacterial replication by binding and denaturing bacterial DNA. Silver ions react with the thiol groups of enzymes, followed by DNA condensation resulting in cell death [28–29].

Blocking of respiration is also a result of the interaction with cell membranes [30].

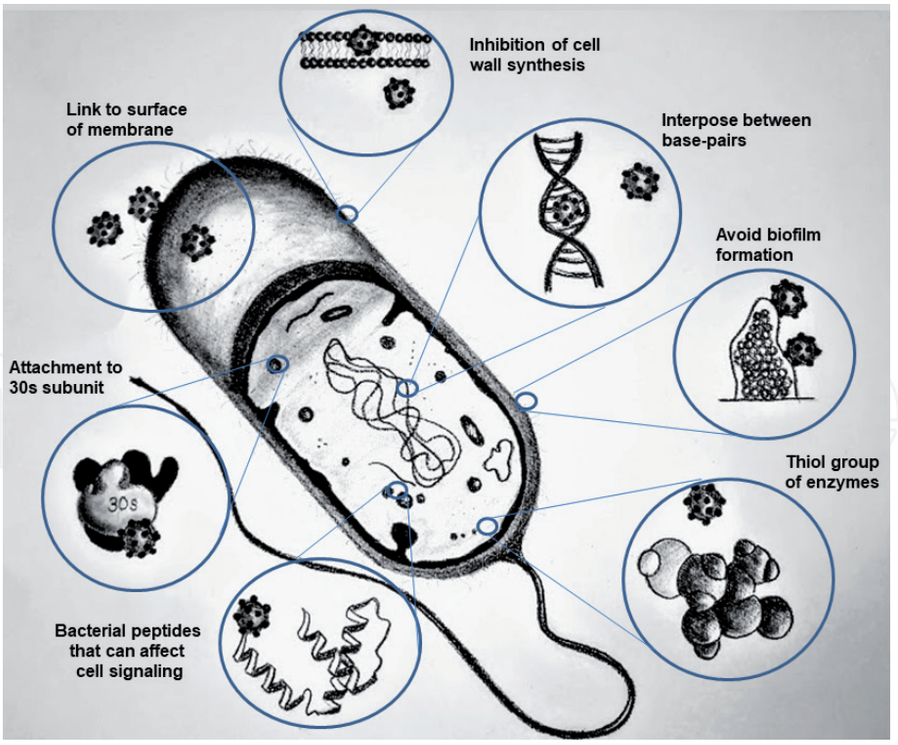

Figure 1.
Mode of action of silver nanoparticles in the bacterial cell.

Disruption of biofilms is another effect of AgNPs. The anti-biofilm action of ~8 nm AgNPs on Gram-negative bacteria has been demonstrated [31]. The outer membrane of Gram-negative contains aquaporins (water-filled channels) which are involved in the transport of Ag ions into the cell where they exert their antibacterial effects [32].

4. Cell status by flow cytometry

Flow cytometry (FCM) is a well-established and powerful analytical tool that has led to many revolutionary discoveries in cell biology and cellular-molecular disease diagnosis and, more recently, has been used to analyze physiological responses of bacteria [33, 34]. In FCM, cells are first introduced to a high-speed (up to 5–20 m/s) laminar flow stream, and after being focused into single file, they are subjected to laser-induced fluorescence, and/or forward and sideways scattered light is detected using photodetector arrays with spectral filters. More recently, FCM has been used to characterize distinct physiological conditions in bacteria including their responses to antibiotics and other cytotoxic chemicals [33]. Once the control of bacterial cells or fungal conidia has been applied, an accurate technique is required to measure the effectiveness of the silver nanoparticles. Flow cytometry is one of the most reliable techniques for detecting and counting living cells and to measure their viability.

When studying response to antibacterial agents such as silver nanoparticles, viability can be evaluated as an indicator of antibiotic susceptibility. There are now reagents available that allow assays of membrane permeability and potential by measuring the production of a fluorescent metabolite from a nonfluorescent precursor [33, 34].

Besides monitoring susceptibility to antibacterial activity, information can be obtained using FCM that can establish mechanisms of antibacterial drug

activity [35–40]. Traditional culture-based techniques cannot do this [41]. The use of fluorescent probes to detect specific cell changes provides a unique tool for interrogating bacteria permeability and changes in membrane potential [42] (**Figure 2**). DNA content and metabolic activity [42] are useful indicators of cell viability and thus of antibiotic susceptibility.

The accuracy of cell counting depends on fluorescent staining. The choice of a fluorescent dye should take into account factors such as membrane permeability, photostability, pH and sensitivity to temperature [43, 44]. The total bacterial count is a key quality criterion for food or beverages [45] and a useful tool for detecting the presence of microbes within matrices. Williams et al. [46] used this technique to detect *E. coli* O157:H7 in raw spinach. The presence of plant pathogens during crop growth has been investigated by several authors. Day et al. [47] used FCM to detect and quantify *Phytophthora infestans* sporangia. A study of colonization of root-associated bacteria in rice was carried out by Valdameri et al. [48]. Otherwise, Golan et al. [49] counted *Pectobacterium carotovorum* subsp. *carotovorum* cells tagged with green fluorescent protein (GFP) in *Ornithogalum dubium* seedlings to detect resistant cultivars. The application of FCM is useful to create the bases for predictive models of spore germination, infection and disease development.

Cell viability assays can distinguish between live and dead cell populations and so correlate with other cell functions or treatments. Many companies offer a wide range of viability dyes, including fixable and non-fixable types and ones specific to bacterial or yeast viability tests. FCM can be applied to monitor the efficacy of treatments to reduce contamination of water [43] and foods and beverages [45, 50] by determining the viability of residual microorganisms. In agriculture FCM can be used to test the effectiveness of antibiotics and antifungals against plant pathogens. The advantage of live FCM cell counts compared to plate counts is that FCM allows the determination of several different morbidity stages between living and dead cells. Some of these are membrane integrity, esterase activity, membrane potential, electron transport, total cells, GFP expression, active/dead, mitochondrial activity, intracellular pH and carotenoid content [51–53].

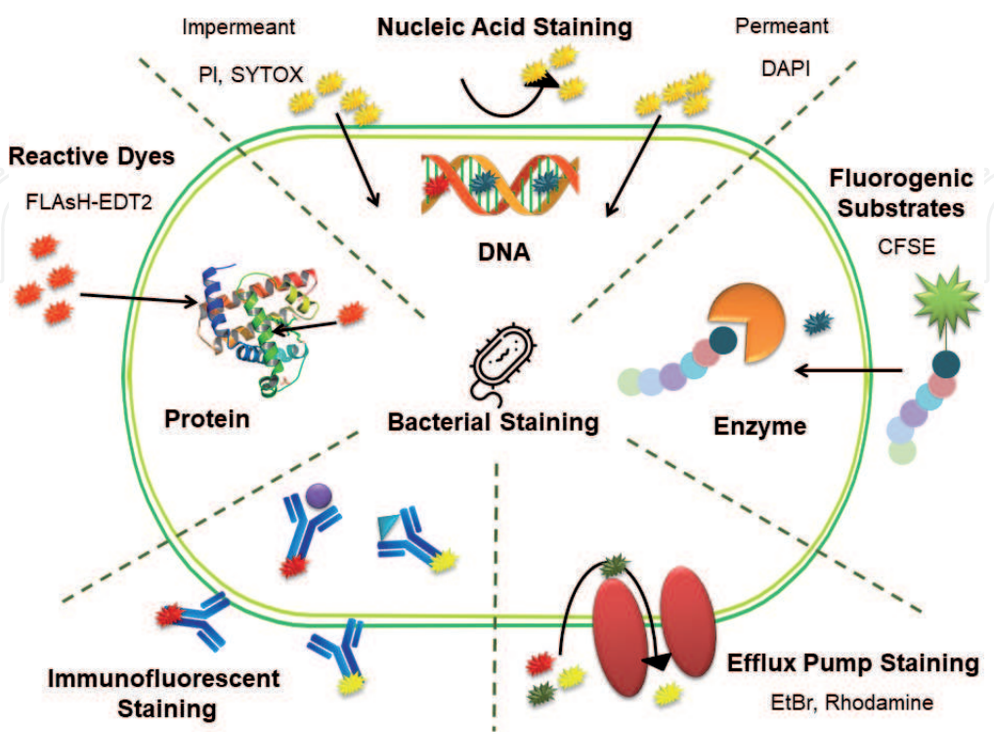

Figure 2.
Fluorescent probes to detect specific bacterial cell changes as an indicator of cell viability.

5. Conclusions

The Food and Agriculture Organization of the United Nations predicts that, globally, about 1.3 billion tons of food is lost per year. A large proportion of this loss is caused by postharvest microbial action. Much of this loss could be averted if more effective procedures and protocols were developed and adopted. Nanotechnology offers a range of novel tools with application in the fight against microbial food spoilage. Silver nanoparticles can act at cell level affecting from the cell wall or finely affecting the DNA. They offer a viable alternative to more traditional methods for the bacterial control. Once bacterial control is achieved using silver nanoparticles, continual bacterial monitoring becomes a critical component of the supply chain. For this, flow cytometry offers an accurate, novel and versatile technology through which to survey bacterial viability in assays of various bacterial control strategies.

Acknowledgements

We are indebted to Carolina Alvarado Gonzalez for the artwork.

Conflicts of interest

The authors declare there is no conflict of interest regarding the publication of this chapter. This chapter has not previously been published and is not being considered for publication elsewhere. The authors certify that neither the manuscript nor its main contents have already been published or submitted for publication in a scientific journal.

Author details

Graciela Dolores Avila-Quezada[1*] and Gerardo Pavel Espino-Solis[2]

1 Faculty of Agrotechnological Sciences, Autonomous University of Chihuahua, Chihuahua, Mexico

2 Faculty of Medicine and Biomedical Sciences, Autonomous University of Chihuahua, Chihuahua, Mexico

*Address all correspondence to: gavilaq@gmail.com

References

[1] Brady CL, Goszczynska T, Venter SN, Cleenwerck I, De Vos P, Gitaitis RD, et al. *Pantoea allii* sp. nov., isolated from onion plants and seed. International Journal of Systematic and Evolutionary Microbiology. 2011;**61**(4):932-937. DOI: 10.1099/ijs.0.022921-0

[2] Bautista-Baños S, Sivakumar D, Bello-Pérez A, Villanueva-Arce R, Hernández-López M. A review of the management alternatives for controlling fungi on papaya fruit during the postharvest supply chain. Crop Protection. 2013;**49**:8-20. DOI: 10.1016/j.cropro.2013.02.011

[3] Liu S, Tang Y, Wang DC, Lin NQ, Zhou JN. Identification and characterization of a new Enterobacter onion bulb decay caused by *Lelliottia amnigena* in China. Applied Microbiology: Open Access. 2016;**2**:1000114. DOI: 10.4172/2471-9315.1000114

[4] Vahling-Armstrong C, Dung JKS, Humann JL, Schroeder BK. Effects of postharvest onion curing parameters on bulb rot caused by *Pantoea agglomerans, Pantoea ananatis* and *Pantoea allii* in storage. Plant Pathology. 2016;**65**(4):536-544. DOI: 10.1111/ppa.12438

[5] García-ÁvilaCDJ,Valenzuela-TiradoGA, Florencio-Anastasio JG, Ruiz-Galván I, Moreno-VelázquezM,Hernández-MacíasB, et al. Organisms associated with damage to post-harvest potato tubers. Revista Mexicana de Fitopatología. 2018;**36**(2):308-320. DOI: 10.18781/R.MEX.FIT.1801-1

[6] Campa-Siqueiros P, Vallejo-Cohen S, Corrales-Maldonado C, Martínez-Téllez MÁ, Vargas-Arispuro I, Ávila-Quezada G. Reduction in the incidence of grey mold in table grapes due to the volatile effect of a garlic extract. Revista Mexicana de Fitopatología. 2017;**35**(3):494-508. DOI: 10.18781/R.MEX.FIT.1707-1 [Accessed: 19 May 2019]

[7] Nunes CA. Biological control of postharvest diseases of fruit. European Journal of Plant Pathology. 2012;**133**(1):181-196. DOI: 10.1007/s10658-011-9919-7 [Accessed: 19 May 2019]

[8] Liu J, Sui Y, Wisniewski M, Droby S, Liu Y. Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. International Journal of Food Microbiology. 2013;**167**(2):153-160. DOI: 10.1016/j.ijfoodmicro.2013.09.004 [Accessed: 19 May 2019]

[9] Combrinck S, Regnier T, Kamatou GPP. In vitro activity of eighteen essential oils and some major components against common postharvest fungal pathogens of fruit. Industrial Crops and Products. 2011;**33**(2):344-349. DOI: 10.1016/j.indcrop.2010.11.011 [Accessed: 19 May 2019]

[10] Miranda-GómezB,García-HernándezA, Muñoz-Castellanos L, Ojeda-Barrios DL, Avila-Quezada GD. Pectate lyase production at high and low pH by *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*. African Journal of Microbiology Research. 2014;**8**(19):1948-1954. DOI: 10.5897/AJMR2014.6765 [Accessed: 19 May 2019]

[11] Guetsky R, Kobiler I, Wang X, Perlman N, Gollop N, Avila-Quezada G, et al. Metabolism of the flavonoid epicatechin by laccase of *Colletotrichum gloeosporioides* and its effect on pathogenicity on avocado fruits. Phytopathology. 2005;**95**(11):1341-1348. DOI: 10.1094/PHYTO-95-1341 [Accessed: 19 May 2019]

[12] Prusky D, Alkan N, Mengiste T, Fluhr R. Quiescent and necrotrophic

lifestyle choice during postharvest disease development. Annual Review of Phytopathology. 2013;**51**:155-176. DOI: 10.1146/annurev-phyto-082712-102349 [Accessed: 19 May 2019]

[13] Torres-Armendáriz V, Manjarrez-Domínguez CB, Acosta-Muñiz CH, Guerrero-Prieto VM, Parra-Quezada RÁ, Noriega-Orozco LO, et al. Interactions between *Escherichia coli* O157:H7 and food plants. Has this bacterium developed internalization mechanisms? Revista Mexicana de Fitopatología. 2016;**34**(1):64-83. DOI: 10.18781/R.MEX.FIT.1507-4 [Accessed: 19 May 2019]

[14] Makavana JM, Makwana PJ, Kukadiya VD, Joshi AM. Post-harvest losses of lemon fruits: An assessment of microbial floral strength during post-harvest handling. International Journal of Current Microbiology and Applied Sciences. 2018;**7**(5):1184-1188. DOI: 10.20546/ijcmas.2018.705.144 [Accessed: 19 May 2019]

[15] Flores-Sánchez JL, Mora-Aguilera G, Loeza-Kuk E, López-Arroyo JI, Domínguez-Monge S, Acevedo-Sánchez G, et al. Yield loss caused by Candidatus *Liberibacter asiaticus* in Persian lime, in Yucatan Mexico. Revista Mexicana de Fitopatología. 2015;**33**(2):195-210. Available from: http://www.scielo.org.mx/pdf/rmfi/v33n2/2007-8080-rmfi-33-02-00195-en.pdf [Accessed: 19 May 2019]

[16] Ávila-Quezada GD, Téliz-Ortiz D, González-Hernández H, Vaquera-Huerta H, Tijerina-Chávez L, Johansen-Naime R, et al. Dinámica espacio-temporal de la roña (*Elsinoe perseae*), el daño asociado a trips y antracnosis (*Glomerella cingulata*) del aguacate en Michoacán, México. Revista Mexicana de Fitopatología. 2002;**20**:77-87. Available from: https://www.researchgate.net/profile/G_D_Avila-Quezada/publication/236569648_Dinamica_espacio-temporal_de_rona_dano_asociado_a_trips_y_antracnosis_en_aguacate/links/568767ab08ae19758397e6a3/Dinamica-espacio-temporal-de-rona-dano-asociado-a-trips-y-antracnosis-en-aguacate.pdf [Accessed: 19 May 2019]

[17] Chudasama B, Vala AK, Andhariya N, Mehta RV, Upadhyay RV. Highly bacterial resistant silver nanoparticles: Synthesis and antibacterial activities. Journal of Nanoparticle Research. 2010;**12**(5):1677-1685. DOI: 10.1007/s11051-009-9845-1

[18] Kim SW, Jung JH, Lamsal K, Kim YS, Min JS, Lee YS. Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. Mycobiology. 2012;**40**(1):53-58. DOI: 10.5941/MYCO.2012.40.1.053 [Accessed: 19 May 2019]

[19] Min JS, Kim KS, Kim SW, Jung JH, Lamsal K, Kim SB, et al. Effects of colloidal silver nanoparticles on sclerotium-forming phytopathogenic fungi. The Plant Pathology Journal. 2009;**25**(4):376-380. DOI: 10.5423/PPJ.2009.25.4.376 [Accessed: 19 May 2019]

[20] Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. Journal of Applied Microbiology. 2012;**112**(5):841-852. DOI: 10.1111/j.1365-2672.2012.05253.x [Accessed: 19 May 2019]

[21] Biao L, Tan S, Wang Y, Guo X, Fu Y, Xu F, et al. Synthesis, characterization and antibacterial study on the chitosan-functionalized Ag nanoparticles. Materials Science and Engineering: C. 2017;**76**:73-80. DOI: 10.1016/j.msec.2017.02.154

[22] Mukherji S, Ruparelia JP, Agnihotri S. In: Cioffi N, Rai M, editors. Nano-Antimicrobials: Progress and

Prospects. Berlin Heidelberg: Springer Verlag; 2012. pp. 225-251. Available from: https://books.google.com.mx/books?hl=es&lr=&id=dRh1fgmnOP0C&oi=fnd&pg=PR3&dq=Mukherji+S,+Ruparelia+JP,+Agnihotri+S.++in+Nano-Antimicrobials:+Progress+and+Prospects,+ed.+N.+Cioffi+and+M.+Rai,+Springer+Verlag,+Berlin+Heidelberg,+2012,+pp.+225%E2%80%93251+&ots=LxBFeqbcCA&sig=c3JIl_O3kpI7jD8ALHvks3u0nwo#v=onepage&q&f=false [Accessed: 19 May 2019]

[23] Agnihotri S, Mukherji S, Mukherji S. Size-controlled silver nanoparticles synthesized over the range 5-100 nm using the same protocol and their antibacterial efficacy. RSC Advances. 2014;**4**:3974-3983. DOI: 10.1039/C3RA44507K [Accessed: 19 May 2019]

[24] Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, et al. Journal of Biological Inorganic Chemistry. 2007;**12**:527-534. DOI: 10.1007/s00775-007-0208-z [Accessed: 19 May 2019]

[25] Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, et al. Nanomedicine: Nanotechnology. Biology and Medicine. 2007;**3**(1):95-101. DOI: 10.1016/j.nano.2006.12.001 [Accessed: 19 May 2019]

[26] Arokiyaraj S, Vincent S, Saravanan M, Lee Y, Oh YK, Kim KH. Green synthesis of silver nanoparticles using *Rheum palmatum* root extract and their antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Artificial Cells, Nanomedicine, and Biotechnology. 2017;**45**(2):372-379. DOI: 10.3109/21691401.2016.1160403 [Accessed: 19 May 2019]

[27] Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for gram-negative bacteria. Journal of Colloid and Interface Science. 2007;**275**:77-82. DOI: 10.1016/j.jcis.2004.02.012 [Accessed: 19 May 2019]

[28] Castellano JJ, Shafii SM, Ko F, Donate G, Wright TE, Mannari RJ, et al. Comparative evaluation of silver-containing antimicrobial dressings and drugs. International Wound Journal. 2007;**4**:14-22. DOI: 10.1111/j.1742-481X.2007.00316.x [Accessed: 19 May 2019]

[29] Eby DM, Luckarift HR, Johnson GR. Hybrid antimicrobial enzyme and silver nanoparticle coatings for medical instruments. ACS Applied Materials & Interfaces. 2009;**1**(7):1553-1560. DOI: 10.1021/am9002155

[30] Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. Advances in Colloid and Interface Science. 2009;**145**(1-2):83-96. DOI: 10.1016/j.cis.2008.09.002 [Accessed: 19 May 2019]

[31] Radzig MA, Nadtochenko VA, Koksharova OA, Kiwi J, Lipasova VA, Khmel IA. Antibacterial effects of silver nanoparticles on Gram-negative bacteria: Influence on the growth and biofilms formation, mechanisms of action. Colloids and Surface B: Biointerfaces. 2013;**02**:300-306. DOI: 10.1016/j.colsurfb.2012.07.039 [Accessed: 19 May 2019]

[32] Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, et al. Silver nanoparticles as potential antibacterial agents. Molecules. 2015;**20**:8856-8874. DOI: 10.3390/molecules20058856 [Accessed: 04 Jul 2019]

[33] Ambriz-AviñaV,Contreras-GarduñoJA, Pedraza-Reyes M. Applications of flow cytometry to characterize bacterial physiological responses. BioMed Research International. 2014. DOI: 10.1155/2014/461941 Accessed: 04 Jul 2019

[34] Lukomska-Szymanska M, Konieczka M, Zarzycka B,

Lapinska B, Grzegorczyk J, Sokolowski J. Antibacterial activity of commercial dentine bonding systems against *E. faecalis*–flow cytometry study. Materials. 2017, 2017;**10**(5):481. DOI: 10.3390/ma10050481 [Accessed: 04 Jul 2019]

[35] Mason DJ, Power EGM, Talsania H, Phillips I, Gant VA. Antibacterial action of ciprofloxacin. Antimicrobial Agents and Chemotherapy. 1995;**39**(12): 2752-2758. DOI: 10.1128/AAC.39.12.2752 [Accessed: 04 Jul 2019]

[36] Roth BL, Poot M, Yue ST, Millard PJ. Bacterial viability and antibiotic susceptibility testing with SYTOX green nucleic acid stain. Applied and Environmental Microbiology. 1997;**63**(6):2421-2431. Available from: https://aem.asm.org/content/63/6/2421.short [Accessed: 04 Jul 2019]

[37] Suller MTE, Stark JM, Lloyd D. A flow cytometric study of antibiotic-induced damage and evaluation as a rapid antibiotic susceptibility test for methicillin-resistant *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy. 1997;**40**(1):77-83. DOI: 10.1093/jac/40.1.77 Accessed: 04 Jul 2019

[38] Mortimer FC, Mason DJ, Gant VA. Flow cytometric monitoring of antibiotic-induced injury in *Escherichia coli* using cell-impermeant fluorescent probes. Antimicrobial Agents and Chemotherapy. 2000;**44**(3):676-681. DOI: 10.1128/AAC.44.3.676-681.2000 [Accessed: 04 Jul 2019]

[39] Novo DJ, Perlmutter NG, Hunt RH, Shapiro HM. Multiparameter flow cytometric analysis of antibiotic effects on membrane potential, membrane permeability, and bacterial counts of *Staphylococcus aureus* and *Micrococcus luteus*. Antimicrobial Agents and Chemotherapy. 2000;**44**(4):827-834. DOI: 10.1128/AAC.44.4.827-834.2000 [Accessed: 04 Jul 2019]

[40] Wickens HJ, Pinney RJ, Mason DJ, Gant VA. Flow cytometric investigation of filamentation, membrane patency, and membrane potential in *Escherichia coli* following ciprofloxacin exposure. Antimicrobial Agents and Chemotherapy. 2000;**44**(3):682-687. DOI: 10.1128/AAC.44.3.682-687 [Accessed: 04 Jul 2019]

[41] Suller MT, Lloyd D. Fluorescence monitoring of antibiotic-induced bacterial damage using flow cytometry. Cytometry. 1999;**35**(3):235-241. DOI: 10.1002/(SICI)1097-0320(19990301)35:3<235::AID-CYTO6>3.0.CO;2-0 [Accessed: 04 Jul 2019]

[42] Laflamme C, Lavigne S, Ho J, Duchaine C. Assessment of bacterial endospore viability with fluorescent dyes. Journal of Applied Microbiology. 2004;**96**(4):684-692. DOI: 10.1111/j.1365-2672.2004.02184.x [Accessed: 04 Jul 2019]

[43] Hammes F, Egli T. Cytometric methods for measuring bacteria in water: Advantages, pitfalls and applications. Analytical and Bioanalytical Chemistry. 2010;**397**(3):1083-1095. DOI: 10.1007/s00216-010-3646-3 [Accessed: 19 May 2019]

[44] Tracy BP, Gaida SM, Papoutsakis ET. Flow cytometry for bacteria: enabling metabolic engineering, synthetic biology and the elucidation of complex phenotypes. Current Opinion in Biotechnology. 2010;**21**(1):85-99. DOI: 10.1016/j.copbio.2010.02.006

[45] Comas-Riu J, Rius N. Flow cytometry applications in the food industry. Journal of Industrial Microbiology & Biotechnology. 2009;**36**(8):999-1011. DOI: 10.1007/s10295-009-0608-x

[46] Williams AJ, Cooper WM, Summage-West CV, Sims LM,

Woodruff R, Christman J, et al. Level 2 validation of a flow cytometric method for detection of *Escherichia coli* O157:H7 in raw spinach. International Journal of Food Microbiology. 2015;**215**:1-6. DOI: 10.1016/j.ijfoodmicro.2015.08.011 [Accessed: 19 May 2019]

[47] Day JP, Kell DB, Griffith GW. Differentiation of *Phytophthora infestans* sporangia from other airborne biological particles by flow cytometry. Applied Environmental Microbiology. 2002;**68**:37-45. DOI: 10.1128/AEM.68.1.37-45

[48] Valdameri G, Kokot TB, Pedrosa FDO, de Souza EM. Rapid quantification of rice root-associated bacteria by flow cytometry. Letters in Applied Microbiology. 2015;**60**(3):237-241. DOI: 10.1111/lam.12351

[49] Golan A, Kerem Z, Tun OM, Luzzatto T, Lipsky A, Yedidia I. Combining flow cytometry and gfp reporter gene for quantitative evaluation of *Pectobacterium carotovorum* spp. *carotovorum* in *Ornithogalum dubium* plantlets. Journal of Applied Microbiology. 2010;**108**(4):1136-1144. DOI: 10.1111/j.1365-2672.2009.04517.x

[50] Hahn MA, Keng PC, Krauss TD. Flow cytometric analysis to detect pathogens in bacterial cell mixtures using semiconductor quantum dots. Analytical Chemistry. 2008;**80**(3):864-872. DOI: 10.1021/ac7018365

[51] Sträuber H, Müller S. Viability states of bacteria-specific mechanisms of selected probes. Cytometry Part A. 2010;**77**(7):623-634. DOI: 10.1002/cyto.a.20920

[52] Freitas C, Nobre B, Gouveia L, Roseiro J, Reis A, da Silva TL. New at-line flow cytometric protocols for determining carotenoid content and cell viability during *Rhodosporidium toruloides* NCYC 921 batch growth. Process Biochemistry. 2014;**49**(4):554-562. DOI: 10.1016/j.procbio.2014.01.022

[53] Linhová M, Branská B, Patáková P, Lipovský J, Fribert P, Rychtera M, et al. Rapid flow cytometric method for viability determination of solventogenic clostridia. Folia Microbiologica. 2012;**57**(4):307-311. DOI: 10.1007/s12223-012-0131-8