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# 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.

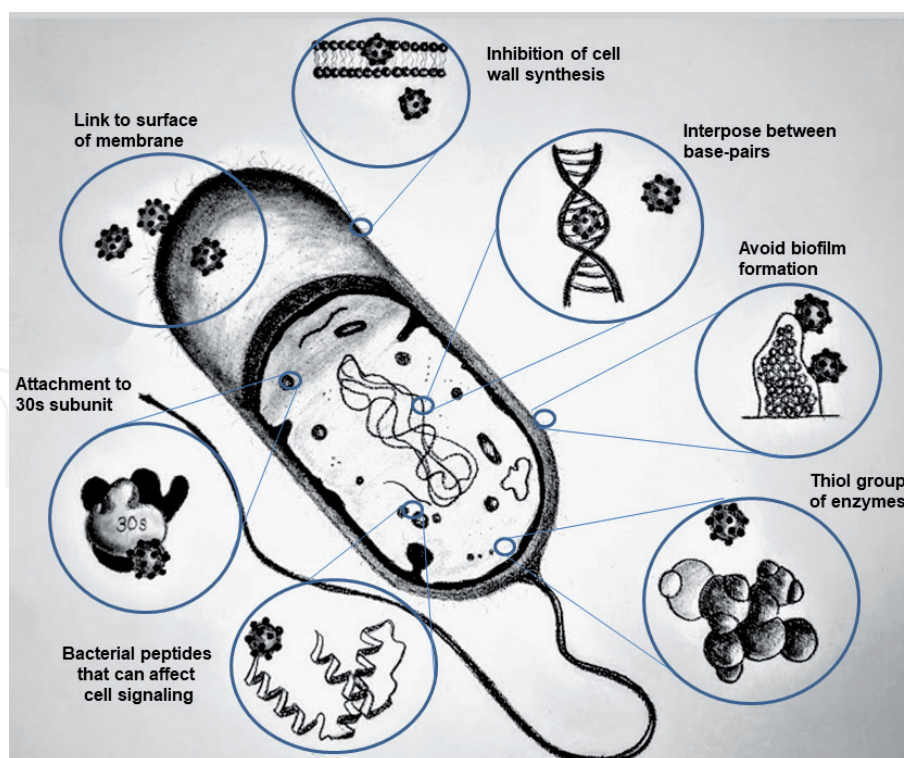
In addition, AgNPs can penetrate bacterial membranes, facilitating internalization. The rupture or perforation of the cell wall is an evidence of internalization of AgNP and of uncontrolled transport through the cytoplasm resulting in cell death [27] (**Figure 1**).

#### **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.

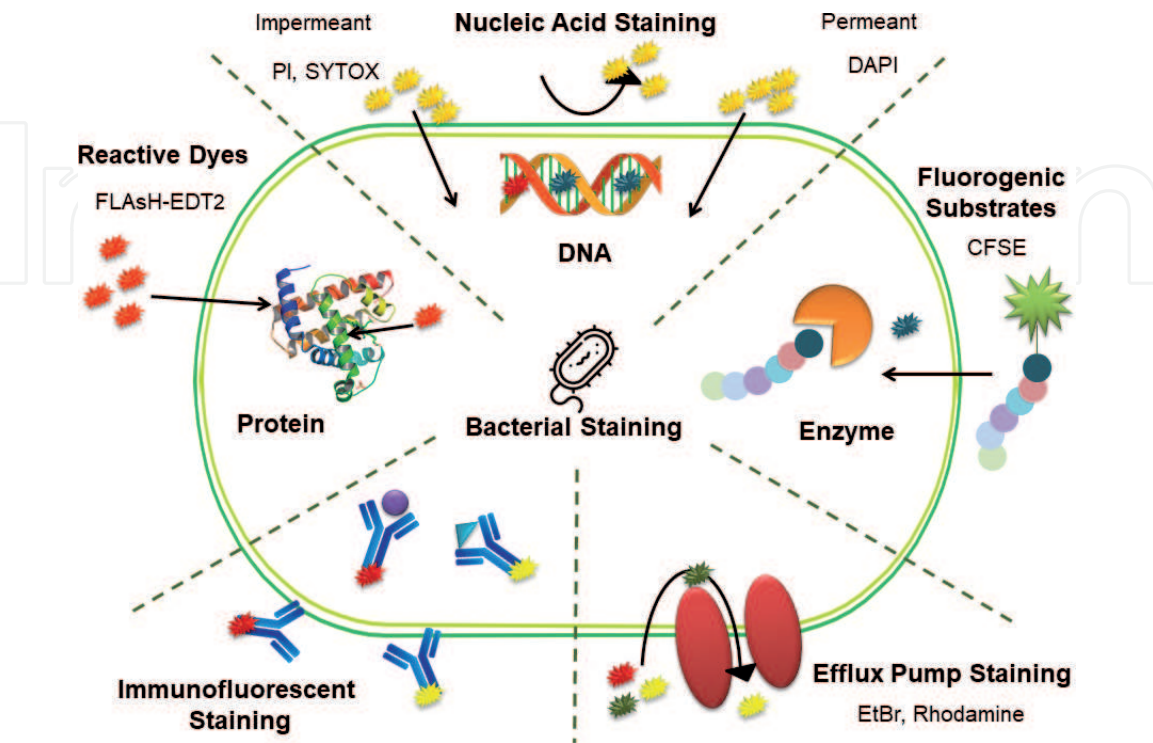
When studying response to antibacterial agents such as silver nanoparticles, viability can be evaluated as an indicator of antibiotic susceptibility. There are now reagents available that allow assays of membrane permeability and potential by measuring the production of a fluorescent metabolite from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

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## 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.

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