Se-Nanoparticles from Bacterial Biotransformation

Subjects: Biology

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Selenium nanoparticles (SeNPs) are gaining importance in the food and medical fields due to their antibacterial properties. The microbial inhibition of these kinds of particles has been tested in a wide range of Gram (+) and Gram (−) pathogenic bacteria. When SeNPs are synthesized by biological methods, they are called biogenic SeNPs, which have a negative charge caused by their interaction between surface and capping layer (bioorganic material), producing their high stability.

Keywords: selenium-nanoparticle ; bacteria ; antibacterial activity ; selenium biotransformation

1. Introduction

Nanoparticles (NPs) are defined as particles with one or more dimensions on the order of 100 nm or less [1][2] and are synthesized in different shapes and sizes through physical and chemical processes. Numerous investigations have demonstrated that NPs have remarkable properties in comparison to their bulk materials [3]. Furthermore, valuable micronutrients have been synthesized as NPs. In this sense, an important element in human nutrition, like selenium, has been prepared as nanoparticles, which have been called selenium nanoparticles (SeNPs).

In the last decade, many investigations have studied the SeNPs properties and have proven that SeNPs show high bioactivities with potential applications in several industries (i.e., food, medicine, or pharmacology) [4].

SeNPs have been synthesized by many processes; however, biotechnological methods have been one of the most important because the production of toxic species is avoided. In fact, it is known that many bacteria are capable of synthesizing SeNPs naturally through detoxification mechanisms [5][6][7], in which salts of selenite and selenate are reduced to non-toxic elemental selenium [8][9][10]. The antimicrobial activity of SeNPs has been studied by a great number of researchers, in which it has been found that SeNPs have a broad range of action against both pathogenic Gram-positive Gram-negative bacteria [4].

2. SeNPs Morphology and the Antimicrobial Activity

In recent years, SeNPs have attracted attention due to their unique antimicrobial properties. They are considered a novel and potential alternative to standard antibiotics, as they have great potential against increasing multidrug resistance in pathogenic bacteria and fungi. The development and synthesis of these nanomaterials for their use as antimicrobials depend directly on some physical and chemical properties, such as concentration, zeta-potential, surface area, size and shape.

2.1. SeNPs Concentration

One of the most important physico-chemical parameters that affect the antimicrobial activity of SeNPs is their concentration. This property is directly related to microbial species due to their different characteristics of the cell surface. Cremonini et al. [11] indicated that the antimicrobial activity of SeNPs synthesized by *Stenotrophomonas maltophilia* and *Bacillus mycoides* inhibited the growth of clinical isolates of *Pseudomonas aeruginosa* at concentrations from 8 to 512 mg/mL but did not inhibit *Candida albicans* and *Candida parapsilosis* species. On the other hand, El-Deeb et al. [12] indicated that 10 µg of SeNPs synthesized by *Providencia vermicola* BGRW had a strong inhibitory effect on the growth of four Gram-positive pathogens (*S. aureus*, *B. Cereus*, methicillin-resistant *S. aureus* and *S. agalactiae*) and *E. coli*. However, most Gram-negative bacteria (*P. aeruginosa*, *Enterobacter* sp., *Enterococcus* sp., *Proteus mirabilis*, *Klebsiella* sp., *Salmonella enteritidis* and *Stenotrophomonas maltophilia*) and *Candida albicans* are not inhibited at this concentration. Greeshma and Mahesh [1] indicated that 400 µg of SeNPs produced by *Bacillus* showed an antibacterial effect on *St. mutans*, *B. cereus*, *E. coli* and *C. albicans*, but it was in the last two that the greatest inhibition was observed.

Likewise, several authors have shown that antibacterial activity depends on the amount of SeNPs. For example, rising from 10 to 15 µg, the inhibition of 4 Gram-positive pathogenic bacteria (14% *S. aureus*, 17% *B. cereus*, 24% *S. agalactaie*, 31% *E. coli* and 37% methicillin-resistant *S. aureus*) was significantly increased. On the contrary, when a concentration between 2.5 and 10 µg/mL of SeNPs is used, there is no antibacterial effect in any of the strains tested in the study by El-Deeb et al. [12]. Comparably, Alam et al. [13] demonstrated complete inhibition in the growth of 5 strains (*E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa*, *K. pneumonia*) when 1 to 10 µg/mL of SeNPs were used, this concentration was calculated as the minimum inhibitory (MIC90) after 4 to 6 h of contact with the bacteria, but by doubling it, and leaving it in contact for 6 h, the SeNPs act as a bactericide.

Similarly, Bharathi et al. [14] reported that antibacterial activity on Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumoniae*) is higher when a concentration of 10 at 100 µg of SeNPs was used. Additionally, these researchers also demonstrated that the bactericidal concentration is specific for each bacterium. They tested the MIC (8 µg SeNP/200 µL) and the minimum bactericidal concentration (CMB, 8 µg SeNP/100 µL) and observed that *P. aeruginosa* showed greater sensitivity to SeNPs compared to *E. coli*, *K. pneumoniae* and *S aureus*.

In the same way, Zhang et al. [15] found that 500 mg/L of SeNPs got a greater antibacterial activity on Gram-negative bacteria than on Gram-positive ones. In this sense, some authors have established stabilized parameters for nanoparticles through the interaction with biological molecules (polysaccharides and proteins) to decrease minimum inhibitory concentrations and increase their activity spectrum. *B. subtilis* was treated with SeNPs stabilized with bovine serum albumin (BSA), D-glucose and soluble starch. Scanning electron microscopy (SEM) analysis revealed morphological changes in bacterial cells treated with all three types of SeNPs compared to untreated *B. subtilis* cells. A considerable reduction in the amount of viable *B. subtilis* was observed in soluble starch-SeNPs, in which cells were shrunken and fragmented, and a polydispersity of cell size was observed. This effect was contrary to that observed in untreated *B. subtilis* [16].

However, in a study carried out with SeNPs stabilized with chitosan, it was shown that antimicrobial activity was effective only against Gram-positive bacteria (*Streptococcus sanguinis*, *Staphylococcus aureus* and *Enterococcus faecalis*) but not against Gram-negative bacteria (*Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *E. coli*), using a MIC in a range of 0.068 to 0.274 mg/mL. Moreover, it was observed that MIC decreases when SeNPs are stabilized with polysaccharides. In addition, the results revealed that MIC of 0.274 mg/mL had a greater bactericidal effect with Gram-positive bacteria after 6 h of contact [17].

2.2. Coating Surface and Charge

The surface charge of nanoparticles is characterized by the zeta-potential, which is another factor that plays an important role in antimicrobial activity since the interaction between nanoparticles and the cell membrane is based on electrostatic adhesion [18]. This interaction is observed because bacteria have specific characteristics that explain their behaviour in contact with SeNPs. For example, both Gram-positive and Gram-negative bacteria have a negatively charged surface [19][20][21][22], which could attract positively charged nanoparticles [23].

Specifically, SeNPs have shown better antimicrobial effectiveness against Gram-positive bacteria than Gram-negative bacteria [17][24][25]. However, the mechanisms for the different effects of SeNPs against bacteria are not entirely clear. Galić et al. [26] designed SeNPs coated with polyvinylpyrrolidone (PVP-SeNPs), poly-L-lysine (PLL-SeNPs) and polyacrylic acid (PAA-SeNPs) to obtain neutral, positively and negatively charged SeNPs, respectively. Antibacterial action of all the studied SeNPs was observed against Gram-positive *S. aureus* (24 h MBC 25–50 mg Se/L), but not against *E. coli* and *S. cerevisiae*. In a similar investigation, Rangrazi et al. [17] showed that positively charged chitosan SeNPs have good antimicrobial activity against Gram-positive bacteria (*Streptococcus sanguinis*, *Staphylococcus aureus* and *Enterococcus faecalis*), but no bactericidal effect was found against Gram-negative bacteria (*Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Escherichia coli*).

In the same way, Filipović et al. [27] stabilized SeNPs with bovine serum albumin (SeNPs-BSA), chitosan (Chit) and glucose (Gluc). -BSA and SeNPs-Chit showed positive potentials (SeNPs-BSA, +27 ± 3; SeNPs-Chit, +24 ± 1) and the SeNPs-Gluc showed negative potential (−45 ± 1). These were tested to inhibit the growth of four Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis* and *Kocuria rhizophila*) and four Gram-negative bacteria (*Escherichia coli*, *Salmonella abony*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*). They observed that zeta-positive SeNPs showed significantly greater antibacterial activity than negatively charged SeNPs, with the exception of those in contact with *Escherichia coli*. This bacterium was inhibited by negatively charged SeNPs at a lower concentration (290 µg/mL) than SeNPs-BSA, which had a positive charge (400 µg/mL). On the other hand, Cremonini et al. [11]

produced two types of SeNPs, which were synthesized by *B. mycoides* SeITE01 and *S. maltophilia* SeITE02. Additionally, a chemically synthesized SeNP was tested. The three SeNPs generated negative zeta-potential between −70 and −80 mV; however, the antimicrobial activity against clinical isolates of *P. aeruginosa* was greater when they used biogenic SeNPs than those chemically synthesized. The authors attributed these differences to the presence of a bacterial protein layer that covers the surface of the biogenic particles and which is related to the zeta-potential.

Several studies attribute the antimicrobial activity of biogenic SeNPs to the presence of a natural covering or capping on their surface, such as polysaccharides, proteins and lipids [4][28][29][30]. The presence of this coating provides them with natural stability, which makes them highly stable, mainly for clinical treatments. Studies on SeNP coating functions have shown that in addition to particles stability, they also affect their zeta-potential.

Recently, Tugarova et al. [31] reported that SeNPs synthesized by *A. brasilense* Sp7 cells are covered by a bioorganic layer that comprises proteins, polysaccharides and lipids, with a significant proportion of ionized carboxylic groups. Sidechains of some amino acid residues and carboxylated polysaccharides typically form these last groups, but they are also responsible for the negative SeNPs' zeta-potentials. This has also been suggested by other researchers, such as Lampis et al. [32], who identified an organic or biomolecular layer composed of carbohydrates, lipids and proteins around the negatively charged SeNPs produced by *Stenotrophomonas maltophilia* SeITE02.

Likewise, Khoei et al. [33] also observed that SeNPs zeta-potential generated by *Burkholderia fungorum* 95 and *Burkholderia fungorum* DBT1 was related to the presence of exopolysaccharides as a component of the organic layer around the SeNPs, suggesting that exopolysaccharides composed of carbohydrates, proteins, and humic-like substances may govern SeNPs' zeta-potential produced by microorganisms. Studies on SeNPs and their charge, measured by zeta-potential, are carried out to establish their stability and antimicrobial effectiveness; that is why the research field is open to expanding the knowledge of this physicochemical relationship.

2.3. Size and Shape

One of the most important physicochemical properties that affect antimicrobial activity is size. Typically, smaller nanoparticles have relatively higher stability and antimicrobial activity than bigger ones. This is because smaller nanoparticles present a greater surface area, which provides superior interaction and intracellular penetration [34][35][36]. In particular, SeNPs with a smaller size than 100 nm biosynthesized by *Stenotrophomonas maltophilia* SeITE02 demonstrated a better antimicrobial effect to inhibit pathogens (*S. aureus*, *E. coli* and *P. aeruginosa*) compared to those with a size between 100 and 400 nm [35].

Similarly, SeNPs obtained extracellularly from *B. licheniformis* with a size between 10 and 50 nm exhibit an antimicrobial effect in the inhibition of foodborne pathogens (*B. cereus*, *E. faecalis*, *S. aureus*, *E. coli* O157: H7, *S. typhimurium*, *S. enteritidis*) at concentrations of 25 µg/mL [34]. In a study developed by Huang et al. [37], the authors reported that SeNPs of 81 nm inhibited completely methicillin-resistant and not methicillin-resistant *Staphylococcus aureus*. Interestingly, SeNPs of 81 nm (25 µg/mL) showed greater antimicrobial activity compared to SeNPs of 124 nm (140 µg/mL), even though the concentration of nanoparticles used was lower. However, when they are proved in their bactericidal activity, the behaviour is different. SeNPs of 43 nm and 81 nm at concentrations of 12.5 µg/mL and 0.78 µg/mL, respectively, showed significant bactericidal activity toward *S. aureus*. In this case, the minimum concentration at which bactericidal effect of SeNPs was lower with the bigger particles.

Recent investigations have reported that antimicrobial activity is also dependent on the relationship between nanoparticle size and nanoparticle coatings, such as chitosan, bovine serum albumin (BSA) and glucose. In general, the highest antimicrobial activity is found in SeNPs associated with chitosan (SeNP-Chit), which showed a significantly larger size and diameter distribution (>100 nm) than SeNPs associated with bovine serum albumin (<100 nm). Although the SeNPs-Chit and those associated with glucose had a similar size, it was the SeNPs-Chit, which showed the highest antimicrobial activity [27].

Regarding the shape of the nanoparticles, it has been demonstrated that it has an influence on antimicrobial activity due to the interaction of the nanoparticle with the cell membrane. In this context, TEM analysis has shown that the most commonly reported form of SeNPs synthesized by bacteria are spherical, and in some cases, nano tubes or nanorods are observed along with spheres [38][39][40][12][41][42]. In contrast, SeNPs of chemical or physical origin has the shape of sheets, plates, tubes, rods, cubes, ribbons and triangles [16]. Nevertheless, spherical particles are more easily internalized within cells compared to large or elongated particles because the spherical form helps to be always in contact with the cell membrane [43]. Despite the relationship between form and interactions with the cell membrane, studies and antimicrobial

activity of SeNPs are very scarce. On the other hand, there are different physicochemical and microbiological parameters that also affect the size and shape of SeNPs at the time of biogenic production.

2.3.1. Physicochemical Parameters That Affect Shape and Size of SeNPs

The uniform distribution of shape and size of SeNPs during bacterial synthesis is influenced by several parameters such as the effect of sodium selenite concentration, pH and temperature in the medium. In the case of sodium selenite concentration, Wadhwani et al. [40] reported that bacteria *Acinetobacter* sp. synthesizes SeNPs with the spherical shape at concentrations of 0.3 to 2.0 mM of Na_2SeO_3. However, rod-like shapes were observed at higher concentrations (2.5 to 4.0 mM Se), which at 3.0 mM were shorter. In the same way, Moreno-Martin et al. [44] observed that the shape depends on the initial selenite concentration in culture media. Sodium selenite concentrations of 100 mg/L generate spherical SeNPs by fermentation of *L. acidophilus* and *L. reuteri*. However, *L. bulgaricus* with this same concentration of selenite synthesizes SeNPs in a star shape. This behaviour is also observed in methicillin-resistant *Staphylococcus aureus*; this bacterium synthesizes spherical SeNPs and nano rod-like structures, while *E. coli*, *S. aureus* and *Pseudomona aeruginosa* showed spherical SeNPs when they were cultured in the presence of 2 mM Na_2SeO_3 [38].

Like the shape, the size of SeNPs synthesized by bacteria is also influenced by selenite concentration. In a cell suspension of *Acinetobacter* sp. SW30 treated with different concentrations of Na_2SeO_3 (0.3, 0.5, 1.0 and 1.5 mM) it is possible to obtain different diameter sizes of SeNPs (126, 96, 113 and 78 nm, respectively). In this experiment, the authors reported the smallest synthesized particles at 1.5 mM of Na_2SeO_3 [40]. Furthermore, Presentato et al. [45] reported that the size and aggregation of SeNPs are strongly influenced by the initial concentration of Na_2SeO_3 in media. The smallest SeNPs (71 and 53 nm) obtained at the lowest concentration (0.5 mM Na_2SeO_3) evolve to form Se-nanorods, which are comparatively larger than those obtained at higher concentrations (2 mM). Other parameters such as pH and temperature in the culture medium for the synthesis of SeNPs have also been studied. However, it has been observed that both parameters are related to the growth conditions of the bacteria and not to the formation of SeNPs, such as in chemical synthesis. For example, it has been reported that temperature for the synthesis of SeNPs of *Acinetobacter* sp. SW30 is at 30 and 37 °C, with the maximum observed synthesis at 37 °C, because the responsible proteins for the reduction of sodium selenite are active only at these temperatures [46][40]. Previous studies by Wang et al. [47] showed that when *B. subtilis* bacterium was subjected to a heat treatment (heating at 100 °C for 1 h) to obtain SeNPs, the colour of the reaction solution did not change, which indicated that high temperatures caused the inactivation of bacteria enzymes and the synthesis of SeNPs was not carried out.

In relation to pH, it has been found that at values of seven and nine, a maximum concentration of SeNPs is synthesized by *Bacillus* sp. EKT1 after 72 h of incubation using selenium dioxide as an inorganic selenium source in the medium [46]. Similarly, in *Bacillus megaterium,* the maximum synthesis of SeNPs is reached at pH 7 and 8, knowing that this microorganism grows under optimal conditions (37 °C, 0.25 mM Na_2SeO_3, 30 h incubation) at slightly alkaline pH [48]. On the other hand, when pH is between 6 and 9 during *Acinobacter* sp. growth, no significant effect was observed on the shape and size of synthesized SeNPs, and the concentration (1.5 and 3.0 nM) did not have a direct effect on these parameters [40].

2.3.2. Microbiological Parameters That Affect SeNPs Size and Shape

Several researchers have shown that SeNPs synthesized by bacteria evolve in size and shape depending on the incubation time. For example, Wang et al. [47] demonstrated that SeNPs synthesized by *B. subtilis* have a spherical shape which becomes elongated as the incubation time elapses. SeNPs synthesized in the first 24 h have a size of 50–150 nm, while those synthesized after 48 h have a diameter close to 400 nm. In another study, it was also observed that at short incubation times, the shape of SeNPs by *Acinetobacter sp.* is spherical in contrast to the elongated shape obtained with longer times (up to 48 h). This effect is attributed to the Ostwald ripening process, which is caused by the high free energy derived from SeNPs [40].

On the other hand, the number and size of SeNPs synthesized by *Stenotrophomonas maltophilia* SeITE02 increase according to the incubation time when 0.5 mM of sodium selenite concentration is in the medium, obtaining SeNPs of 150 nm in diameter after 24 h of incubation. This time is related to the end of the exponential phase, while in the late stationary phase (after 48 h), this size can be between 100 to 300 nm [32]. Similarly, Lampis et al. [49] determined that SeNPs produced by *Bacillus mycoides* at 6 h of incubation have an average diameter of 50 to 100 nm, and after 48 h, their dimensions range between 50 and 400 nm. This behaviour has also been observed in *Pantoea agglomerans*, *Bacillus subtilis* and *Shewanella* sp when SeNPs are produced [50][51][47].

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