https://dx.doi.org/10.21577/0103-5053.20230062

J. Braz. Chem. Soc., Vol. 34, No. 7, 897-905, 2023

Review

Biogenic Silver Nanoparticles Capped with Proteins: Timed Knowledge and Perspectives

Nelson Durán,[*,a,b] *Wagner J. Fávaro,*[a] *Silvana Alborés,*[c] *Thyerre S. da Costa*[d] *and Ljubica Tasic*[*,d]

[a]*Laboratório de Carcinogenese Urogenital e Imunoterapia, Departamento de Biologia Estrutural e Funcional, Universidade Estadual de Campinas (UNICAMP), 13083-970 Campinas-SP, Brazil*

[b]*Nanomed, Centro de Ciências Naturais e Humanas (CCNH), Universidade Federal do ABC (UFABC), 09218-580 Santo André-SP, Brazil*

[c]*Laboratory of Microbiology, Department of Bioscience, Facultad de Química, Universidad de la República (UDELAR), Montevideo, Uruguay*

[d]*Laboratório de Química Biológica, Instituto de Química, Universidade Estadual de Campinas, 13083-970 Campinas-SP, Brazil*

Biogenic silver nanoparticles are synthesized through silver(I) reduction, which is promoted by biomolecules available in the biological world and mostly obtained from plant extracts, fungal bioproduction, and some bacteria. The exact mechanisms accounting for such oxidoreduction processes are not fully known. However, some studies have already mentioned oxidoreductases, cofactors (nicotinamide adenine dinucelotide hydrogen (NADH), dihydroflavine-adenine dinucleotid ($FADH_2$)), and phenolic compounds, as the main reductive species engaged in the formation of silver(0) and silver nanoparticles (silver NPs) synthesis. Biosynthesis is a one-pot process that leads to stable silver NP colloids that, regarding their size, shape, and uniformity, can be successfully controlled; and show great stability when one takes into account their surface capping by some biomolecules that as well take part in their synthesis. Although great efforts have been made to feature capping biomolecules and their interactions with silver NP surfaces, knowledge of the quantity (exact number *per* cm^2) and type of biomolecules that cap or surround silver NPs remains limited. The literature provides detailed information on protein capping, but it still shows gaps regarding many aspects of fine biophysical protein featuring. The reason why certain proteins prefer to interact with silver NP surface and form chemical bonds, whereas others rather have intermolecular interaction with the first layer of proteins remains unknown. Assessing capping proteins' involvement in the bioactivity of biogenic silver NPs is another relevant research field. Certain proteins enhance bioactivity of silver NPs and lower toxicity; however, the way antimicrobial processes benefit from protein capping is yet to be discovered. Finally, biogenic silver NPs can be found both in the environment and in water; moreover, their additional activity and behavior must be known or, at least, hypothesized.

Keywords: biogenic synthesis, silver nanoparticles, protein capping, bioactivity, stability

1. Introduction

Metallic nanoparticles (NPs) show special qualities, as well as different chemical and physical properties than those of bulk-like metals; among them, one finds tiny size and a high surface/volume ratio. These features make them efficient in acting as nanomedicines. Accordingly, all the herein collected data were indicative of silver nanoparticles as particularly useful for biomedical applications. This profile likely results from their antimicrobial activity on Gram-negative and Gram-positive bacteria, fungi, and viruses. Among so many different synthetic methods, the biological ones seem to be more advantageous than conventional chemical or physical strategies.[1-3] Biogenic synthesis methods based on the use of plants or enzymes or extracts of microorganisms are eco-friendly, safe, and economically feasible and allow the fabrication of

*e-mail: nduran@unicamp.br; ljubica@unicamp.br
Editor handled this article: Jaísa Fernandes Soares

NPs with size control and morphology.[3-10] It is important highlighting that proteins, sugars, and other biological molecules not only act as reducing and adjuvant agents but also as covering agents to stabilize NPs in all biogenic methods.[2,3,11-16] Assumingly, biological molecules in the surface layer capping the metallic core of NPs (biogenic) can improve their activities with other biological systems and enhance interactions with cells or organisms.[17,18] The current expansion of the production of NPs has been inevitably leading to their discharge into the environment; thus, it is essentially describing the corona on biogenic metallic nanoparticles and assesses these molecules' involvement in the synthetic procedure, as well as their importance in modulating the stability of the particle and/or reactivity.

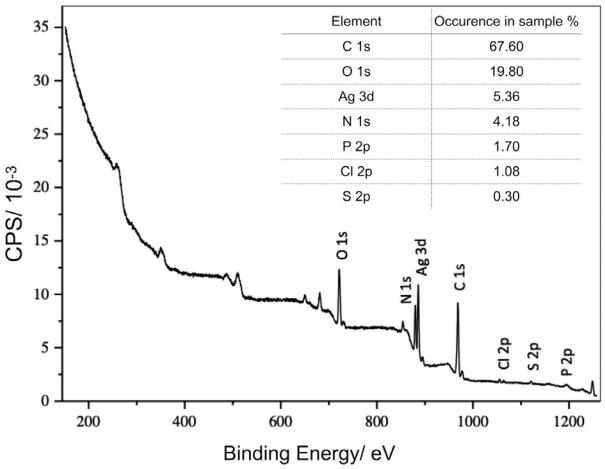


Figure 1. X-ray photoelectron spectroscopy (XPS) of silver NP sample in low resolution (0-1300 eV).

2. Capping Proteins on NPs

2.1. Fungal biogenic nanoparticles

Using fungi for silver nanoparticle production is the way to improve production capacity due to large amounts of secreted substances. These bioactive substances, mainly proteins, play a dual functional role as reducing and stabilizing agents.[19] It is interesting observing that, despite the importance of capped proteins for the biogenic synthetic procedure of metal nanoparticles, there are only a few in-depth studies about capping in these nanoparticle types. According to Durán *et al*.,[20] UV-Vis spectra from the reaction medium of *Fusarium oxysporum* silver NPs' biogenic synthesis showed an absorption band at 265 nm (excitations band of tyrosine and tryptophan residues, in proteins). This finding was indicative of protein release in the *F. oxysporum* medium; moreover, assumingly, nitrate reductase activity was involved in the mechanism likely accounting for reducing metal ions. Furthermore, the observed proteins seemed to be involved in capping of nanoparticles, as suggested by Ahmad *et al*.[21] In addition, fluorescence spectra of fungal filtrate of *Fusarium oxysporum* 07SD showed an emission band centered at 340 nm. The emission band indicated that proteins bound to the surface of the nanoparticle were in their native forms.[22] Furthermore, the nanoparticles were monitored through X-ray photoelectron spectroscopy (XPS, Figure 1). As can be noted, Figure 1 shows the presence of carbon (C), oxygen (O), silver (Ag), and nitrogen (N) in higher concentrations than phosphorus (P), chlorine (Cl), and sulfur (S) (insert in Figure 1). As expected, Ag (from nanoparticles), C, O, N, and S (associated with proteins) were found in the sample, and P may be related to post-translational modifications, such as phosphorylation (addition of a phosphate group to an amino acid in the protein chain).

Gade *et al*.[23] showed that S-atoms in *Aspergillus niger* silver NPs samples were indicative of proteins capping the nanoparticles. Elgorban *et al*.[24] confirmed the presence of a covering layer onto AgNPs through transmission electron microscopic (TEM) analysis. Devi and Joshi[25] published the synthesis of silver NPs by *Aspergillus niger*, *Aspergillus tamarii*, and *Penicillium ochrochloron*. Such way produced silver NPs, besides plasmonic bands, contained the peaks at 280 nm, which were attributed to amino acid residues secreted by fungi such as tryptophan and tyrosine. Additional research is needed to assess the compositions and bio-activity of silver NPs due to the relevance of biogenic nanoparticles' capping.[26]

The biological synthesis of silver nanoparticles (size 16-20 nm) by fungal species *Macrophomina phaseolina* has shown natural protein capping of nanoparticles. Proteins in cell filtrate were characterized by numerous bands with molecular masses ranging from 50 and 116 kDa (Figure 2, lane 2). Treatment applied to silver nanoparticles based on 1% sodium dodecyl sulfate (SDS) in boiling water detached the capped protein(s) from the NPs. Analysis by the SDS-polyacrilamide gel electrophoresis (PAGE) procedure showed that the boiled sample presented a strong band at 85 kDa (Figure 2, lane 4), but the same outcome was not observed in the non-boiled sample (Figure 2, lane 3), which recorded results similar to that of protein bands in cell filtrate (Figure 2, lane 2). This 85-kDa protein is likely to behave as a capping material and provide stability to silver NPs. The authors have justified this finding by stating that it was also observed in the literature.[27,28]

Fungal isolate *Penicillium shearii* AJP05 generated protein-capped silver NPs (size 6-15 nm). The UV-Vis spectrum of uncapped silver NPs and the supernatant of

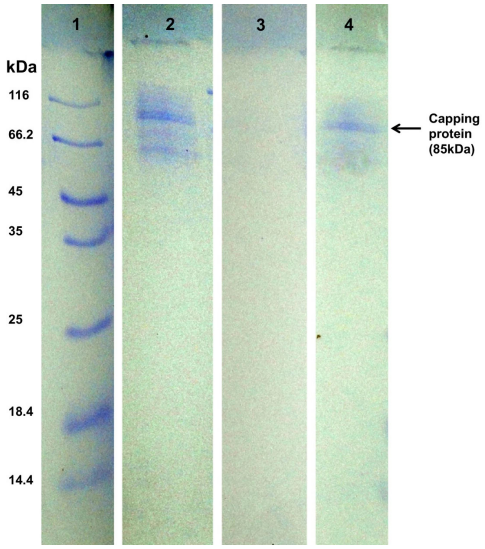


Figure 2. SDS-PAGE analysis of capping proteins around the silver nanoparticles. Lane 1, molecular size marker; lane 2, extracellular proteins in the cell filtrate; lane 3, nanoparticles loaded without boiling showed no protein band; and lane 4, nanoparticles after boiling with 1% SDS loading buffer showed a major 85-kDa capping protein (figure from reference 29 with CC-BY attribution).

SDS-treated ones have evidenced peaks at 446 and 280 nm, respectively. The band of capped silver NPs showed a peak at approximately 280 nm, and a band at 466 nm; this finding pointed toward the presence of protein acting as covering material on the surface of the nanoparticles. The Fourier transform infrared (FTIR) spectrum showed characteristic bands at wavenumbers 1637 cm^{-1} (bending vibration) and 3268 cm^{-1} (stretching vibrations) typical for the amide I bond. Capping materials were isolated after they were treated with 1% SDS solution in boiling water and resolved on SDS-PAGE (12% resolving gel). Based on the results, the SDS-treated sample presented three bands at 65, 55, and 50 kDa, and they were observed in the extracellular cell-free filtrate of *P. shearii*. These proteins acted as coating material and they may have given stability to silver NPs.[30,31]

Guilger-Casagrande *et al.*[31] reported the biological synthesis of silver NPs by using *Trichoderma harzianum* filtrate, based on enzyme production stimulation through the presence of *Sclerotinia sclerotiorum* cell wall; this procedure designated AgNP-TSC (57.02 nm; zeta potential of −18.70 mV) - the procedure unstimulated designated AgNP-TC (81.84 nm, zeta potential of −18.30 mV). The typical FTIR bands of silver NPs were 1637 and 1535 cm^{-1}. These findings were designated as amides I and II that, in their turn, were characteristic of protein presence. Based on the SDS-PAGE assay, proteins in the fungal filtrate were preserved in capping, and bands were observed at 40 and 36 kDa. The aforementioned authors suggested that these bands corresponded to the molecular weight of chitinase and β-1,3-glucanase enzymes deriving from *T. harzianum.*

Rodrigues *et al.*[32] in similar outcomes, found the same protein bands (at 75, 122, 191, and 328 kDa) for the filtrate solution and capping of NPs produced by *Aspergillus tubingensis*. These results corroborated filtrate proteins' participation in the NPs' synthetic procedure as well as their retention in the layer surrounding the nanoparticles. A similar analysis was carried out by Jain *et al.*[27] who found bands at 35 and 32 kDa in silver NPs synthesized by *Aspergillus flavus*.

Ballottin *et al.*[33] prepared *F. oxysporum* silver NPs (40 nm in size, and zeta potential of −35 mV). FTIR results have shown that proteic residues bound to silver NPs did not exhibit relevant secondary structure deviation due to interaction with silver NPs, or when these nanoparticles covalently bonded to them. These outcomes were in compliance with results in other publications in the literature[34-36] and with the fact that protein residues interact with the silver NPs over free amino groups, such as cysteine residues and/or charge interactions through carboxyl groups.

Raman spectroscopy is an important technique to indicate presence of capping proteins at the surface of the investigated silver NPs.[36,37] The nature of silver NPs opens room for the surface-enhanced Raman scattering (SERS) phenomenon, which accounts for a local amplifier effect. This effect enhances the Raman signal of molecular fragments close to the surface of nanoparticles; this process helps assess the stabilizing agent's nature.[38]

Raman bands designated to amides vibrations in amino acid side chains, and other properties of C–H stretching and stretching vibrations of –SH groups were observed in silver NPs samples, just as shown in previous research.[36,39,40] Raman spectra gave information about whether the protein binding to the surface of nanoparticles took place through free amino groups at 243 cm^{-1} or through cysteine residues at 231 cm^{-1}.[41] Raman spectra monitoring during silver NPs synthesis showed increased intensity in the band at 243 cm^{-1}, in unwashed silver NPs, and this finding is indicative of the free $–NH_2$ protein group's interaction with the surface of silver nanoparticles since this band is connected to the N–Ag vibrations. The rise in band intensity due to Ag–S vibrations was lower than that at 243 cm^{-1}. These outcomes are likely denotative that the amount of S–Ag bonds was less than the amount of N–Ag bonds formed in unwashed silver NPs.[33]

Penicillium expansum biogenic NPs were characterized through confocal Raman microscopy, which showed that this biogenic synthesis has produced silver NPs bound with N or O atoms that came from the protein matrix. In addition, there were some bands connected with vibrational modes that correspond to the C–C stretching of D- and G-bands

of the carbonaceous matrix, this finding was attributed to the oxidized organic matter.[38] Estevez *et al.*[19] reported similar results for *Phanerochaete chrysosporium* biogenic NPs (*Pch*NPs). Interestingly, bands at 1474, 1443, 1298, 1022, 1100, 1011, 845, and 815 cm^{-1} could be attributed to L-alanine and L-valine amino acids involved in biogenic capping of *Pch*NPs. These nanoparticles were conjugated to an antimicrobial peptide, nisin (nis) (*Pch*NPs@nis), in a previous study. Raman spectra of bioconjugate *Pch*NPs@nis showed differences in intensity, shift, or new Raman bands appearance in comparison to unconjugated nisin and *Pch*NPs spectra. The Raman analysis has pointed out changes in *Pch*NPs@nis spectra, and this finding suggests molecular interaction between amino acids present in biogenic *Pch*NPs and nisin capping, a fact that points towards *Pch*NPs@nis nano bioconjugates' formation.[42]

Studies focused on characterizing *Aspergillus tubingensis* biogenic silver NPs (35 nm in size zeta potential of +8.48 mV) were carried out by Ballotin *et al*.[36] Their stabilization promoted by extracellular fungal proteins was also assessed. Silver NPs presented a surface plasmonic resonance band at 440 nm, which is typical of silver NPs, as well as the band at 280 nm due to electronic excitation in tyrosine tryptophan, and/or in phenylalanine residues, in proteins from fungi. Based on Raman spectroscopy, proteins from fungi were covalently bonded to silver NPs, mostly through S–Ag bonds, by cysteine residues (HS–), as well as too a few N–Ag bonds from H_2N– groups (Figure 3).[36]

The existence of protein-capping at the surface of the studied silver NPs,[39,40,42] is shown in Figure 3. This technique allowed observing proteins associated with the surface of nanoparticles through free amino groups or via S–H from cysteine. The Raman spectrum (λ_{exc} at 633 nm) presented minor vibrational advice related to materials found on surfaces of silver nanoparticles. The broadband close to 214 cm^{-1} likely belonged to the Ag–Cl vibration (due to chloride ion) and Ag–S vibration overlap, since it indicates an association between superficial silver and the HS-group from coated-proteins' cysteine. The excitation band of 785 nm was strong and corresponded to the amide III and amide I bands of the adsorbed proteins. In addition, bands at 1338 and 1768 cm^{-1}, respectively, presented results similar to those observed through FTIR, which were assigned to amides.[41] Bands at 1138 and 1120 cm^{-1} were assigned to N–CH bending and C–CH stretching modes, respectively, and those at 1234 cm^{-1}, to vibrations in antiparallel β-sheet in the structure of the protein.[41] The amide II mode was observed close to 1635 cm^{-1}. The lack of H–CS bending between 800 and 900 cm^{-1} reinforced the assumption that protein binding to surfaces of silver NPs took place through

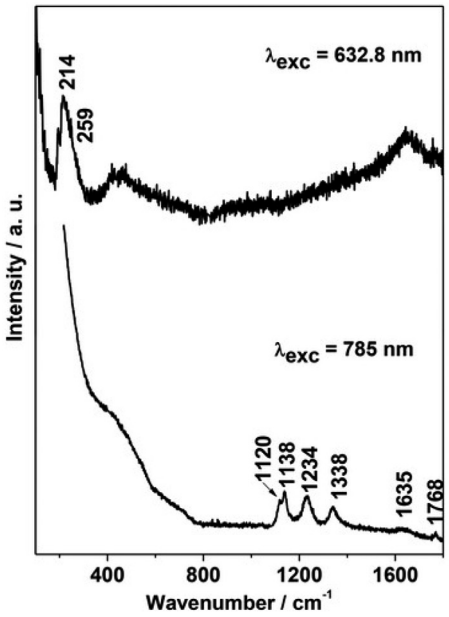


Figure 3. Raman spectra of AgNPs were recorded with laser excitations of 632.8 and 785 nm. The main wavenumbers discussed further in the text are pointed out (figure from reference 36 with CC-BY attribution).

–SH groups. Proteins found in silver NPs were covalently bound to silver, mainly via S–Ag bonds, besides some other interactions between proteins.[35] It was also observed that supramolecular interactions resulted from charged (electrostatic) and probably through other protein-protein interaction mechanisms. Moreover, proteins that remained free on the surface of silver NPs likely use hydrogen bonds to other protein residues or water, thus contributing to the covering layer around the silver nanoparticles and, then, expanding the hydrodynamic diameter of the particles. The FTIR technique was not efficient in detecting relevant changes in the secondary structure. Eight proteins in dispersion of silver nanoparticles were identified through mass spectrometry analysis, namely: 2 glycosidases, 2 glucoamylases, 1 acid phosphatase, 1 serine carboxypeptidase, 1 glucanosyltransferase, and 1 previously considered hypothetical protein. Authors[35] suggested that these proteins played relevant roles in fungal metabolic pathways; thus, proteins important for fungi were also involved in biogenic production and stabilization of silver nanoparticles, as aforementioned.

Surface corona presents specific characteristics in metallic NPs, such as bio-identity, conformational and structural properties, colloidal stability, and chemical platform for any possible modification. Investigations focused on biogenic NPs have been assessed, but not deep enough, since these studies are extremely limited. An interesting strategy to overcome this limitation was applied by Rajput and McDermott[43] who performed a detailed assessment involving both dispersion analyses *in situ* of

silver NPs synthesized by *Fusarium oxysporum* and surface corona desorbing. The authors applied a series of orthogonal techniques for characterization purposes and evidenced that the protein surface corona of biogenic silver NPs comprises a thin mixed layer of peptides and carbohydrates. They pointed out that the way taken to analyze the protein surface corona inherent to biogenic silver NPs must be thoroughly analyzed to allow reproducible and critical interpretations of the properties of silver NPs and bioactivity.

2.2. Bacterial biogenic nanoparticles

Despite a large number of reports on protein-coated biogenic nanoparticles synthesized by fungi, similar biological syntheses performed by bacteria have also been reported. The biological synthesis of silver NPs obtained from Gram-negative *Pseudomonas aeruginosa* was reported by Quinteros *et al.*[15] Electrophoresis analysis and nano high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) reports followed by bioinformatics systems were carried out to characterize proteins from *P. aeruginosa* supernatant. Several proteins were found in capping related to silver NPs, such as azurin and alkyl hydroperoxide reductase. Alkyl hydroperoxide reductase is assumingly responsible for silver ions reduction into Ag^0 in silver NPs. Azurin interacts with silver nanomaterials. This protein may account for the formation of silver NPs, as well as for changes in reactivity of silver NPs, a fact that has implications for targeting, uptake, and cytotoxicity of AgNPs. In addition, some bacterial structural proteins identified in the analysis could be involved in stability of NPs.[15]

Wypij *et al.*[44] reported the biogenic synthesis of silver NPs from actinobacterial strain SH11 (16 nm in size, zeta potential of –17.1 mV). Applying the SDS-PAGE separation method of the cell-free supernatant processed with silver ions, in their study, showed two proteins that were not observed in the control sample (autolysate, Figure 4).

These protein bands presented molecular mass corresponding to 48.4 (01)[45] and 38.2 (2)[46,47] kDa, respectively. The LC-MS/MS analysis applied to these two bands after trypsin digestion produced tryptic peptides that were characterized based on the *m/z* value. The two treated bands were identified as peptides based on the significant homology to proteins from outer membrane porin D (OprD) family porin determined in *Pseudomonas fluorescens* (isoelectric point (pI) of 5.73) and from the same protein (porin) found in *Cupriavidus* sp. HMR1 (pI of 9.1), respectively.

However, it is curious that the aforementioned authors did not explain the origin of peptides identified on the surface of silver NPs since they were not found in the biomass actinobacterial strain autolysate.[44]

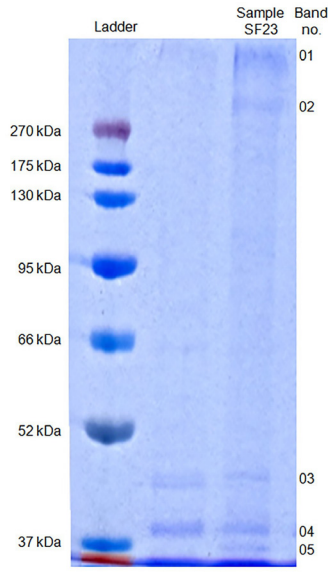


Figure 4. SDS-PAGE of proteins associated with AgNP biosynthesized from actinobacterial strain SH11 (figure from reference 44 with CC-BY attribution).

3. Antimicrobial Activity, Toxicity, Applications

Several studies have shown that protein capping in biogenic silver NPs likely plays a relevant role in the antimicrobial and cytotoxic action of these nanoparticles. The appearance of natural capping proteins rules out capping post-synthesis steps that are essential for most nanoparticle applications in the medical field.[29] The antimicrobial activities of eco-friendly silver NPs synthesized from *Solanum trilobatum* extract and chemically synthesized silver nanoparticles were compared by Ramanathan *et al.*[48] Their results have evidenced no visible inhibition zone for chemically synthesized silver nanoparticles against *Escherichia coli*. Based on this finding, the antimicrobial action of eco-friendly obtained silver NPs is much higher than the antimicrobial properties of chemically synthesized silver nanoparticles. Thus, it can be said that the attendance of starch and protein components in plant extracts guarantees higher stability and antimicrobial actions in biosynthesized silver NPs.[48]

The nanoclusters are shielded by several surface-capping such as ligands, deoxyribonucleic acid (DNA), and proteins, among others. Different surface capping can influence their reactive oxygen species (ROS) formation ability, which can straight intervene in their antimicrobial efficiency. A previous report[48] has shown that negatively charged nanoclusters, with negative zeta potential value

(ζ = –36 mV), would significantly induce ROS generation in both *E. coli* and *S. aureus*. However, they found that induced ROS production decreased when they showed an intrinsic lower charge, with a negative zeta potential value (ζ = –21 mV). Metal nanoclusters (NC) are also inclined to agglomerate in large nubs when they face a problem similar to that of NPs.[49] These binders play a significant role in the nanocluster formation process by regulating the shape, size, and properties.[50]

Chowdhury *et al.*[29] applied scanning electron microscopy (SEM) to detect capping in biogenic silver NPs against multidrug-resistant bacteria. Similar outcomes were reported by Estevez *et al*.[51] who used biogenic silver nanoparticles from the fungal species *Phanerochaete chrysosporium* in multi-resistant *Salmonella enteritidis*. Albeit all strains were unaffected by, at least, one antibiotic, the majority of them were responsive to biogenic silver NPs. In addition, the NPs demonstrated high bactericidal action on three multi-resistant *Salmonella typhimurium* strains. Confocal Raman microscopy (CRM) and atomic force microscopy (AFM) studies demonstrated changes in cellular composition and morphology, cytoplasmic content, and integrity loss of cell envelope.

The antimicrobial activity of silver NPs from *Penicillium expansum* was evaluated and interactions between them and *E. coli* were assessed through TEM and ESEM (environmental scanning electron microscopy), which demonstrated silver NPs binding to the surface of bacteria and bacterial cell membrane rupture. The CRM approach confirmed that TEM results recorded for silver NPs led to damage in bacterial and fungal cells, which, in their turn, resulted in crucial changes to polysaccharides, proteins, lipids, and nucleic acids.[38]

Silver NPs obtained from *Pseudomonas aeruginosa* presented effective antimicrobial action on different bacterial species in comparison to the silver NPs chemically produced. Distinct proteins were characterized in capping related to silver NPs (e.g., reductase and azurin). Proteins identified likely exerted their high antimicrobial activity through silver NPs.[15]

Biogenic silver NPs from *Pseudomonas putida* (P-NPs) were assayed on pathogenic *P. aeruginosa* PAO1, and biogenic nanoparticles from *Escherichia coli* (E-NPs) were assayed against pathogenic *E. coli* UTI 89.[52] The minimum bactericidal concentration (MBC) of P-NPs on *P. aeruginosa* exhibited a 1 μg mL^{-1} value, and that of E-NPs against *E. coli* UTI 89 was higher at 8 μg mL^{-1}. In these cases, MBC values were higher than the ones recorded for green silver NPs synthesized in organisms unrelated to target pathogens accessible in the literature. Chandrasekharan *et al*.[53] recorded higher MBC values for *Gmelina arborea*-mediated silver NPs against *P. aeruginosa* (90 μg mL^{-1}) and *E. coli* (40 μg mL^{-1}). Based on these data, silver NPs synthesized in microorganisms nearly related to the target pathogen can be higher efficient, and it suggests that, assumingly, the biological corona composition plays important role in the antimicrobial action and in the existence of a possible silver NPs mechanism.

Protein-capped and uncapped silver NPs from *Trichoderma harzianum* were compared for their antifungal potential against *Sclerotinia sclerotiorum*. Silver NPs produced by *Trichoderma harzianum* filtrate, with enzyme generation stimulated by the assistance of *Sclerotinia sclerotiorum* cell wall designated AgNP-TSC (57.02 nm, zeta potential of −18.70 mV), but nanoparticles produced without such a stimulation designated AgNP-TC (81.84 nm, zeta potential of −18.30 mV).[31] Capped nanoparticles exhibited large inhibitory potential on *S. sclerotiorum*; the best results were observed when AgNP-TSC was used; uncapped nanoparticles, in their turn, were ineffective to do so.

The existence of protein capping material is advantageous due to its actions as an anchoring layer for pharmaceutical or genetic products to be carried into human cells[54,55] showed that the existence of nontoxic protein cover also enhances the uptake and retention within human cells.

Capping proteins from biogenic silver NPs synthesized by *F. oxysporum* (40 nm in size, and zeta potential of –40 mV) seem to be related to their genotoxicity. The process of washing the silver NPs did not bring significant differences associated with the mitotic index in the *Allium cepa* assay, but unwashed NPs exhibited an important damage index in the damage analysis. A similar outcome was found in the Comet test, in which unwashed NPs were genotoxic and the washed ones were not.[56]

Trichoderma harzianum biogenic nanoparticles did not show a difference in cytotoxicity in comparison to capped and uncapped NPs; however, the larger genotoxicity of uncapped NPs was found in cell lines.[57]

4. Final Remarks

The aims of the present literature review were to show biogenic silver nanoparticle synthesis and knowledge of reducing and stabilizing agents, capping agents, and their roles in enhancing the stability and bioactivity of these remarkable nanomaterials. Biogenic silver NPs are formed by a very complex mechanism that involves more than one biomolecule. This process leads to the formation of stable colloids presenting metallic cores and shells made of some proteins from the starting biomaterial. Protein capping interacts with metallic surfaces through

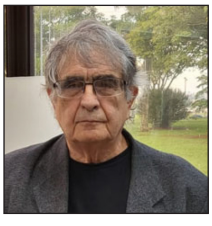
chemical bonds; they engage sulfur (cysteine) and free amino groups from the first protein layer in the shells. Not all proteins from the biogenic material used in the synthesis interact with the surface of NPs in these ways. In addition, the second protein layer was assumingly formed through intermolecular interactions covering the proteins already linked to the surface of silver NPs, and proving sometimes thick corona surrounding stabilizers. These cappings play significant roles when biogenic silver NPs interact with biological samples; the literature shows many examples that enhanced nanomaterial bioactivity could be simply explained by protein capping interventions and involvement in recognition of microbes (based on the posterior devastation effects of metallic core), by full bacterial inhibition and inhibition of fungal growth or viral replication. Finally, some studies in the literature have investigated the toxic effects of these nanomaterials, and the fully capped silver NPs were the least harmful ones because silver NPs showed higher toxicity when the intermolecularly linked protein layer was removed. Research in this exciting field showed great progress, yet there are some gaps to be addressed in order to successfully characterize all biosynthesis steps and the actions of such powerful antimicrobials.

Acknowledgments

At this opportunity, we would like to acknowledge National Institute for Bioanalytics (INCTBio) sponsored by the São Paulo Research Foundation (FAPESP) grant No. 2014/50867-3, and National Council for Scientific and Technological Development (CNPq) grant No. 465389/2014-7.

Author Contributions

All the authors cooperated in the formal analysis, writing, reviewing, and editing of the original draft. Ljubica Tasic, Silvana Alborés, Wagner J. Fávaro, and Nelson Durán cooperated in the conceptualization and final revision of the manuscript; Ljubica Tasic and Nelson Durán edited the final version of the manuscript.



Nelson Durán *is a Professor at the Biology Institute at UNICAMP. He received his PhD at the University of Puerto Rico (USA). In 1978, he joined the Chemistry Institute of UNICAMP. He was the Coordinator of several nanotechnological networks. Member of Brazilian-NanoReg-European Community. He was a member of the Consultant in the Commision of Nanotechnology Area in the Science and Technology of Brazilian Ministry. He published 594 papers, 56 book chapters, 6 books, 229 proceedings, 80 patents, and 2 marks.*

Wagner José Fávaro *is an Associate Professor of Anatomy, Pathology and Oncology at the University of Campinas, UNICAMP (Brazil). He received his Master, PhD and Post-doctorate at UNICAMP. Coordinator of Urogenital Carcinogenesis and Immunotherapy Laboratory (LCURGIM) at UNICAMP. He is an international member of the American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology (ESMO). Founding Partner of NANO-IMMUNOTHERAPY PHARMA LTDA, a company dedicated to developing medicines for human and veterinary uses. Responsible for developing the OncoTherad immunotherapy, which has therapeutic applications in several tumors.*

Silvana Alborés *is Full Time-Professor of Microbiology at the Faculty of Chemistry at UDELAR and Researcher in the ANII and PEDECIBA in Uruguay. She received her PhD in Chemistry in 2011. She did postdoctoral internships at UNICAMP and CNPEM (Brazil), UNIZAR (Spain), the University of Cordoba (Argentina), and UMN (USA). She is the PhD supervisor and head of postgraduate and undergraduate courses in Nanotechnology and Microbiology. She leads research lines in Nanobiotechnology and Antimicrobials. She was responsible for projects with national and international collaboration. She has reviewed numerous articles in international peer-reviewed scientific journals and published 7 manuscripts in the last 3 years.*

Thyerre Santana da Costa *has a bachelor's degree in Chemistry, awarded with the Lavoisier prize from CRQ-IV as the best student in his class at UNICAMP. He is currently in a PhD program at the Institute of Chemistry of UNICAMP with a scholarship from FAPESP under supervision from Prof Dr Ljubica Tasic, with the research project in the area of biophysics and biochemistry.*

Ljubica Tasic *is a chemist (PhD), and an associate professor at the Department of Organic Chemistry at the University of Campinas. She leads the Biological Chemistry Laboratory with research interests in biological chemistry, biochemistry, nanochemistry, and metabolomics. Some of the group's scientific goals are to discover in which way biogenic silver nanoparticles are produced, understand the way they are stabilized with the biomolecular capping, and in what way they interact with the cells.*

References

1. Spagnoletti, F. N.; Kronberg, F.; Spedalieri, C.; Munarriz, E.; Giacometti, R.; *J. Environ. Manage*. **2021**, *297*, 113434. [Crossref]
2. Tasic, L.; Stanisic, D.; Martins, L. G.; Cruz, G. C. F.; Savu, R. In *Sustainable Nanotechnology for Environmental Remediation*, vol. 1, 1st ed.; Koduru, J. R.; Karri, R. R.; Mubarak, N. M.; Bandala, E. R., eds.; Elsevier, 2022, ch. 3. [Crossref]
3. Sidhu, A. K.; Verma, N.; Kaushal, P.; *Front. Nanotechnol.* **2022**, *3*, 801620. [Crossref]
4. Durán, N.; Marcato, P. D.; de Conti, R.; Alves, O. L.; Costa, F. T. M.; Brocchi, M.; *J. Braz. Chem. Soc*. **2010**, *21*, 949. [Crossref]
5. Durán, N.; Silveira, C. P.; Durán, M.; Martinez, D. S. T.; *J. Nanobiotechnol*. **2015**, *13*, 55. [Crossref]
6. Durán, M.; Silveira, C. P.; Durán, N.; *IET Nanobiotechnol.* **2015**, *9*, 314. [Crossref]
7. Sanguiñedo, P.; Fratila, R. M.; Estevez, M. B.; de la Fuente, J. M.; Grazú, V.; Alborés, S.; *Nano Biomed. Eng*. **2018**, *10*, 156. [Crossref]
8. Ahmad, S.; Munir, S.; Zeb, N.; Ullah, A.; Khan, B.; Ali, J.; Bilal, M.; Omer, M.; Alamzeb, S.; Salman, S. M.; Ali, S.; *Int. J. Nanomed*. **2019**, *14*, 5087. [Crossref]
9. Xiao, C.; Li, H.; Zhao, Y.; Zhang, X.; Wang, X.; *J. Environ. Manage*. **2020**, *275*, 111262. [Crossref]
10. Bahrulolum, H.; Nooraei, S.; Javanshir, N.; Tarrahimofrad, H.; Mirbagheri, V. S.; Easton, A. J.; Ahmadian, G.; *J. Nanobiotechnol.* **2021**, *19*, 86. [Crossref]
11. Durán, N.; Marcato, P. D.; Durán, M.; Yadav, A.; Gade, A.; Rai, M.; *Appl. Microbiol. Biotechnol*. **2011**, *90*, 1609. [Crossref]
12. Singh, P.; Kim, Y.-J.; Zhang, D.; Yang, D.-C.; *Trends Biotechnol*. **2016**, *34*, 588. [Crossref]
13. Mohamed, A. A.; Fouda, A.; Abdel-Rahman, M. A.; Hassan, S. E. D.; El-Gamal, M. S.; Salem, S. S.; Shaheen, T. I.; *Biocatal. Agric. Biotechnol.* **2019**, *19*, 101103. [Crossref]
14. Khoshnamvand, M.; Huo, C.; Liu, J.; *J. Mol. Struct.* **2019**, *1175*, 90. [Crossref]
15. Quinteros, M. A.; Bonilla, J. O.; Alborés, S. V.; Villegas, L. B.; Paez, P. L.; *Colloids Surf., B* **2019**, *184*, 110517. [Crossref]
16. Prajapati, A. K.; Mondal, M. K.; *J. Environ. Manage*. **2021**, *290*, 112615. [Crossref]
17. Akther, T.; Mathipi, V.; Kumar, N. S.; Davoodbasha, M.; Srinivasan, H.; *Environ. Sci. Pollut. Res.* **2019**, *26*, 13649. [Crossref]
18. Rai, M.; Bonde, S.; Golinska, P.; Trzcinska-Wencel, J.; Gade, A.; Abd-Elsalam, K. A.; Shende, S.; Gaikwad, S.; Ingle, A. P.; *J. Fungi* **2021**, *7*, 139. [Crossref]
19. Estevez, M. B.; Raffaelli, S.; Mitchell, S. G.; Faccio, R.; Alborés, S.; *Molecules* **2020**, *25*, 2023. [Crossref]
20. Durán, N.; Marcato, P. D.; Alves, O. L.; De Souza, G. I. H.; Esposito, E.; *J. Nanobiotechnol*. **2005**, *3*, 8. [Crossref]
21. Ahmad, A.; Mukherjee, P.; Senapati, S.; Mandal, D.; Khan, M. I.; Kumar, R.; Sastry, M.; *Colloids Surf., B* **2003**, *28*, 313. [Crossref]
22. Kumar, C. V.; McLendon, G. L.; *Chem. Mater.* **1997**, *9*, 863. [Crossref]
23. Gade, A. K.; Bonde, P.; Ingle, A. P.; Marcato, P. D.; Durán, N.; Rai, M. K.; *J. Biobased Mater. Bioenergy* **2008**, *2*, 243. [Crossref]
24. Elgorban, A. M.; Aref, S. M.; Seham, S. M.; Elhindi, K. M.; Bahkali, A. H.; Sayed, S. R.; Manal, M. A.; *Mycosphere* **2016**, *7*, 844. [Crossref]
25. Devi, L. S.; Joshi, S. R.; *J. Microsc. Ultrastruct*. **2015**, *3*, 29. [Crossref]
26. Guilger-Casagrande, M.; de Lima, R.; *Front. Bioeng. Biotechnol*. **2019**, *7*, 287. [Crossref]
27. Jain, N.; Bhargava, A.; Majumdar, S.; Tarafdar, J. C.; Panwar, J.; *Nanoscale* **2011**, *3*, 635. [Crossref]
28. Chitra, K.; Annadurai, G.; *J. Nanostruct. Chem.* **2013**, *3*, 9. [Crossref]
29. Chowdhury, S.; Basu, A.; Kundu, S.; *Nanoscale Res. Lett.* **2014**, *9*, 365. [Crossref]
30. Fageria, L.; Pareek, V.; Dilip, R. V.; Bhargava, A.; Pasha, S. S.; Laskar, I. R.; Saini, H.; Dash, S.; Chowdhury, R.; Panwar, J.; *ACS Omega* **2017**, *2*, 1489. [Crossref]
31. Guilger-Casagrande, M.; Germano-Costa, T.; Pasquoto-Stigliani, T.; Fraceto, L. F.; de Lima, R.; *Sci. Rep*. **2019**, *9*, 14351. [Crossref]
32. Rodrigues, A. G.; Ping, L. Y.; Marcato, P. D.; Alves, O. L.; Silva, M. C. P.; Ruiz, R.; Melo, I. S.; Tasic, L.; de Souza, A. O.; *Appl. Microbiol. Biotechnol*. **2013**, *97*, 775. [Crossref]
33. Ballottin, D.; Fulaz, S.; Cabrini, F.; Tsukamoto, J.; Durán, N.; Alves, O. L.; Tasic, L.; *Mat. Sci. Eng. C* **2017**, *75*, 582. [Crossref]
34. Käkien, A.; Ding, F.; Chen, P.; Mortimer, M.; Kahru, A.; Ke, P. C.; *Nanotechnology* **2013**, *24*, 345101. [Crossref]
35. Banerjee, V.; Das, K. P.; *Colloids Surf., B* **2013**, *111*, 71. [Crossref]

36. Ballottin, D.; Fulaz, S.; Souza, M. L.; Corio, P.; Rodrigues, A. G.; Souza, A. O.; Gaspari, P. M.; Gomes, A. F.; Gozzo, F.; Tasic, L.; *Nanoscale Res. Lett.* **2016**, *11*, 313. [Crossref]
37. Fu, F.-N.; de Oliveira, D. B.; Trumble, W. R.; Sarkar, H. K.; Singh, B. R.; *Appl. Spectrosc.* 1994, *48*, 1432. [Crossref]
38. Estevez, M. B.; Mitchell, S. G.; Faccio, R.; Alborés, S.; *Mater. Res. Express* **2019**, *6*, 1250. [Crossref]
39. Pelton, J. P.; McLean, L. R.; *Anal. Biochem.* **2000**, *277*, 167. [Crossref]
40. Tuma, R.; *J. Raman Spectrosc.* **2005**, *36*, 307. [Crossref]
41. Thomas Jr., G. J.; *Annu. Rev. Biophys. Biomol. Struct.* **1999**, *28*, 1. [Crossref]
42. Zimet, P.; Valadez, R.; Raffaelli, S.; Estevez, M. B.; Pardo, H.; Alborés, S.; *Chemistry* **2021**, *3*, 1271. [Crossref]
43. Rajput, S.; McDermott, M.; *ChemRxiv* **2020**. [Link] accessed in April 2023
44. Wypij, M.; Jedrzejewski, T.; Ostrowski, M.; Trzcinska, J.; Rai, M.; Golinska, P.; *Molecules* **2020**, *25*, 3022. [Crossref]
45. Lecoutere, E.; Verleyen, P.; Haenen, S.; Vandersteegen, K.; Noben, J.-P.; Robben, J.; Schoofs, L.; Ceyssens, P.-J.; Volckaert, G.; Lavigne, R.; *MicrobiologyOpen* **2012**, *1*, 169. [Crossref]
46. Hu, Y.-Y.; Cai, J.-C.; Zhou, H.-W.; Zhang, R.; Chen, G.-X.; *Front. Microbiol.* **2015**, *6*, 784. [Crossref]
47. Wypij, M.; Jedrzejewski, T.; Trzcinska-Wencel, J.; Ostrowski, M.; Rai, M.; Golinska, P.; *Front. Microbiol.* **2021**, *12*, 632505. [Crossref]
48. Ramanathan, S.; Gopinath, S. C. B.; Anbu, P.; Lakshmipriya, T.; Kasim, F. H.; Lee, C.-G.; *J. Mol. Struct.* **2018**, *1160*, 80. [Crossref]
49. Rosli, N. A.; Teow, Y. H.; Mahmoudi, E.; *Sci. Technol. Adv. Mater.* **2021**, *22*, 885. [Crossref]
50. Xie, Y.-P.; Shen, Y.-L.; Duan, G.-X.; Han, J.; Zhang, L.-P.; Lu, X.; *Mater. Chem. Front.* **2020**, *4*, 2205. [Crossref]
51. Estevez, M. B.; Casaux, M. L.; Fraga, M.; Faccio, R.; Alborés, S.; *Front. Bioeng. Biotechnol.* **2021**, *9*, 644014. [Crossref]
52. Singh, P.; Mijakovic, I.; *Biomedicines* **2022**, *10*, 628. [Crossref]
53. Chandrasekharan, S.; Chinnasamy, G.; Bhatnagar, S.; *Sci. Rep*. **2022**, *12*, 156. [Crossref]
54. Hu, C. M. J.; Zhang, L.; Aryal, S.; Cheung, C.; Fang, R. H.; Zhang, L.; *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 10980. [Crossref]
55. Rodriguez, P. L.; Harada, T.; Christian, D. A.; Patano, D. A.; Tsai, R. K.; Discher, D. E.; *Science* **2013**, *339*, 971. [Crossref]
56. Lima, R.; Feitosa, L. O.; Ballottin, D.; Marcato, P. D.; Tasic, L.; Durán, N.; *J. Phys.: Conf. Ser.* **2013**, *429*, 012020. [Crossref]
57. Guilger-Casagrande, M.; Germano-Costa, T.; Bilesky-Jose, N.; Pasquoto-Stigliani, T.; Carvalho, L.; Fraceto, L. F.; de Lima, R.; *J. Nanobiotechnol*. **2021**, *19*, 53. [Crossref]

Submitted: December 22, 2022
Published online: April 28, 2023