UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO
DEPARTAMENTO DE CIÊNCIAS BIOLÓGICAS

INGRID AUGUSTO

ALTERAÇÕES ULTRAESTRUTURAIS EM BACTÉRIAS EXPOSTAS A NANOPARTÍCULAS DE OURO SINTETIZADAS COM EXTRATO DE *Virola oleifera*

VITÓRIA
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Trabalho de Conclusão de Curso apresentado ao Departamento de Ciências Biológicas da Universidade Federal do Espírito Santo, como requisito parcial para obtenção do grau de Bacharel em Ciências Biológicas.

Orientador: Prof.º Marco Cesar Cunegundes Guimarães

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Aprovada em 27 de Novembro de 2015.

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EPIGRAFE

“We'll have more power in the volume of a sugar cube than exists in the entire world today.”

Ralph Merkle

RESUMO

Síntese de nanopartículas com propriedades antibacterianas tem grande potencial para o desenvolvimento de novas aplicações biomédicas. Nanopartículas de ouro (AuNPs) são conhecidas por terem efeitos inibitórios e bactericidas, mas existem poucos dados disponíveis sobre os efeitos em patógenos humanos das nanopartículas de ouro sintetizadas por rota verde. Neste trabalho de conclusão de curso, avaliou-se a atividade antibacteriana de AuNPs sintetizadas a partir de extrato de *Virola oleifera* contra dois modelos de bactérias multirresistentes, a Gram positiva *Staphylococcus aureus* e a Gram negativa *Escherichia coli*. Os resultados mostraram que AuNPs foram mais eficazes contra o patógeno Gram positiva com atividade bacteriostática contra *Staphylococcus aureus*, em comparação com bactérias Gram negativas. Encontrou-se também danos na parede celular, e aumento de sua espessura e alteração na estrutura da membrana celular em *S. aureus* exposta a AuNPs. Além disso, sugere-se a alteração na pressão de turgência como mecanismo para a inibição do crescimento. Todo o trabalho é exposto em formato de artigo científico de acordo com as normas do periódico *Nanomedicine: nanotechnology, biology and medicine.*

Palavras-Chave: Nanopartículas de ouro, síntese verde, ultraestrutura, *S. aureus, E. coli.*

LISTA DE SIGLAS E ABREVIATURAS

AuNPs – Nanopartículas de ouro

MDR – Bacterias multiresistentes

MIC – Concentração mínima inibitória (MIC)

MET – Microscópio Eletrônico de Transmissão

MEV – Microscópio Eletrônico de Varredura

OD – Densidade óptica

PG – Peptidoglicano

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SUMÁRIO

1. INTRODUÇÃO

Cada vez mais a Ciência tem se aprofundado acerca dos sistemas biológicos e tem-se deparado com estruturas cada vez menores. Partindo da escala micro, com células, a estruturas nanométricas, como organelas e até sendo capaz de manipular átomos e moléculas, como o próprio DNA. Nas últimas décadas, a chamada nanotecnologia tem desenvolvido novas ferramentas e metodologias, envolvendo estruturas em escala nano, para investigação e transformação de sistemas biológicos (PATIL et al., 2012). Dentre essas ferramentas estão as nanopartículas, estruturas menores que 100 nm que se apresentam em diversos formatos como esferas, discos hexagonais e nanobarras; além de exibirem propriedades química e físicas distintas de seu material de origem (LOVE et al., 2005; MOGHIMI et al., 2005; MORITZ; GESZKE-MORITZ, 2013).

A síntese de nanopartículas ocorre por duas formas: *top-down*, na qual as nanoestruturas são produzidas por meios físicos; e *botton-down* na qual são produzidas por meios químicos (KUBIK et al., 2005). Atualmente prevalece a síntese por meio químico, principalmente por reações químicas de oxi redução por via aquosa utilizando-se de um agente redutor (NARAYANAN; SAKTHIVEL, 2011; MARANGONI, 2012). Normalmente também são utilizados agentes estabilizadores (também chamados de passivadores), que são moléculas usadas para evitar a agregação das nanopartículas, formando uma cobertura sobre a superfície das mesmas, impedindo o contato entre elas. Além de importantes no controle do tamanho, os passivadores também podem funcionalizá-las (SHON; CHOO, 2003).

Entretanto, todos os agentes redutores tradicionalmente usados, como borohidreto e citrato, são tóxicos, inflamáveis e ambientalmente danosos por liberarem resíduos nocivos (LEE et al., 2015). Alternativamente, técnicas de síntese verde na qual utiliza-se de agentes redutores naturais, desde de organismos simples como bactérias a eucariotos complexos, tem sido desenvolvidas (IRAVANI, 2011). Dentre eles destaca-se a utilização de extratos vegetais por dispensarem o cuidado de manutenção da estrutura celular, requerida por outros organismos, e por permitir a produção em alta escala de nanopartículas (MUTHUVEL et al., 2014). Além disso, extratos de plantas são aos mesmo tempo agentes redutores (levando a formação da nanoparticula), agentes estabilizadores (impedem a agregação das nanopartículas) e em alguns casos atuam na funcionalização das nanopartículas

(IRAVANI, 2011; NARAYANAN; SAKTHIVEL, 2011; SADEGHI et al., 2015).

Recentemente nas áreas de medicina, odontologia, farmácia e biologia têm sido utilizadas nanopartículas metálicas e oxido metálicas de prata, ouro, cobre, oxido cobre, zinco, oxido zinco, entre outras (MORITZ; GESZKE-MORITZ, 2013). As nanopartículas de ouro (AuNPs) apresentam a vantagem de serem de um material inerte, resistente a oxidação e não tóxico para humanos (BADWAIK et al., 2012; BINDHU; UMADEVI, 2014). Diversos estudos vem apontando o uso de nanopartículas de ouro em aplicações biomédicas como na regulação genica, quimioterapia, e distribuição de medicamentos (LEE et al., 2015). Mas, é seu potencial antibacteriano que tem despertado o interesse de muitos pesquisadores. Já foi estabelecido a atividade antibacteriana contra diferentes patógenos incluindo bactérias multirresistentes (LOK et al., 2006; TRAN et al., 2013; BINDHU; UMADEVI, 2014; HWANG et al., 2015). Bactérias multiresistentes são definidas como patógenos que apresentam resistência a dois ou mais antibióticos aos quais normalmente são considerados susceptíveis (COUTO, 2003). O desenvolvimento de novos antibióticos e novas estratégias de combate, como as nanopartículas, é de fundamental importância devido ao aumento na frequência de bactérias multirresistentes nas comunidades e em hospitais em todo o mundo (FISCHBACH; WALSH, 2010).

Assim, visando encontrar alternativas para combater a resistência bacteriana foi desenvolvida no Laboratório de Ultraestrutura Celular Carlos Alberto Redins uma rota verde para síntese de nanopartículas de ouro (MILANEZE et al., 2014). Dados preliminares indicam que estas nanopartículas apresentam potencial antibacteriano contra bactérias gram-positivas. Porém, para entender como as nanopartículas podem ser usadas no combate a bactérias é importante a total compreensão de seu mecanismo e formas de interação com a mesmas. Desta forma este trabalho de conclusão de curso propõe uma análise ultraestrutural para verificar o potencial antibacteriano de nanopartículas de ouro biossintetizadas com extrato de *Virola oleífera* e compreender as alterações morfológicas promovidas assim como seu possível mecanismo.

Este trabalho foi estruturado no formato de artigo científico seguindo as normas de publicação da revista científica *Nanomedicine: nanotechnology, biology and medicine* na qual será inicialmente submetido. Assim, o artigo foi subdividido

nas categorias a*bstract*, *background, methods, results, discussion,* e *references*. As normas podem ser encontradas em detalhes na seção Apêndice.

2. OBJETIVOS

2.1 OBJETIVO GERAL

Pesquisar se nanopartículas de ouro sintetizadas a partir do extrato da planta *Virola oleífera* provocam alterações morfológicas em bactérias Gram positivas e Gram negativas.

2.2 OBJETIVOS ESPECÍFICOS

- Síntese de nanopartículas de ouro (AuNP's) utilizando extrato vegetal de *Virola oleifera* como agente redutor;
- Investigar uma possível atividade antibacteriana das nanopartículas sintetizadas frente a bactérias Gram positiva *Staphylococcus aureus* e Gram negativa *Escherichia coli;*
- Verificar as alterações estruturais promovidas pela interação com as nanopartículas nos diferentes grupos de bactérias;
- Propor o mecanismo de ação das nanopartículas de ouro biossintetizadas de *Virola oleifera* contra bactérias;

3. JUSTIFICATIVA

A nanotecnologia tem influenciado recentemente diversas áreas, incluindo a biomédica, com promissores avanços no desenvolvimento de diagnósticos, tratamentos e medicamentos. Por isso explorar este “nano-universo” se torna necessário e de fundamental importância para aumentar nossa compreensão acerca das potencialidades e limitações de aplicação das nanopartículas. É importante não apenas encontrar diferente formas de síntese verde, como a aqui utilizada, como encontrar seus possíveis mecanismos de ação e efeitos em sistemas biológicos.

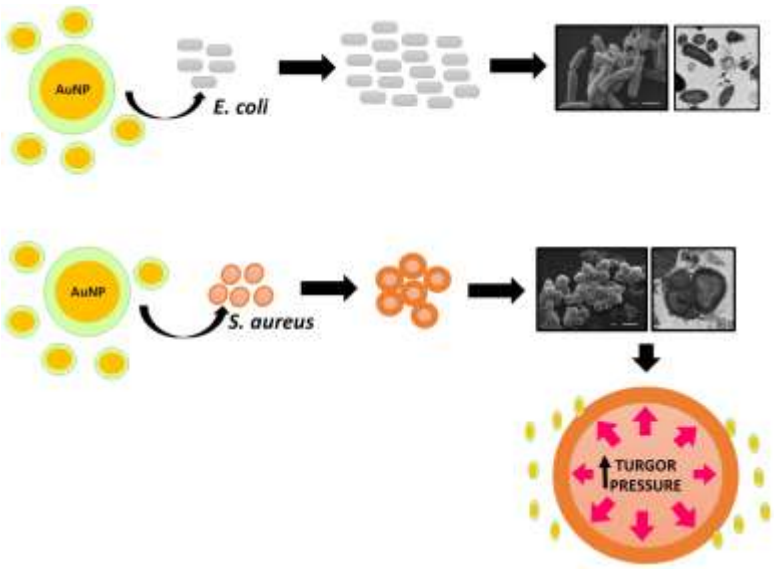
Assim este trabalho irá contribuir para a compreensão do mecanismo de ação de nanopartículas de ouro e seus efeitos em bactérias fornecendo dados que podem ser utilizados no aperfeiçoamento das mesmas e no desenvolvimento de novos

antibióticos.

4. ARTIGO

Ultrastructural changes in bacteria exposed to gold nanoparticles synthesized with *Virola oleifera*

ABSTRACT



Synthesis of nanosized particles with antibacterial properties has great potential in the development of new biomedical applications. Gold nanoparticles (AuNPs) are known to have inhibitory and bactericidal effects, but restricted information is available on the effects of green gold nanoparticles on human pathogens. In this article, we evaluated the antibacterial activity of AuNPs synthesized from *Virola oleifera* extract against two model multidrug resistant bacterias, the Gram positive *Staphylococcus aureus* and the Gram negative *Escherichia coli*. Results showed that AuNPs were more effective against Gram-positive pathogen with significant bacteriostatic activity against *Staphylococcus aureus* as compared with Gram negative bacteria. Also we showed that AuNPs damaged cell wall, which showed increased thickness and affected the cell membrane structure of *Staphylococcus aureus*. Furthermore, we suggest the disturbing in turgor pressure as mechanism to growth inhibition in gram positive

bacteria. In summary, our data indicates AuNPs as a promising model for the design of novel antibacterial agents.

Keywords: Gold nanoparticles, green synthesis, ultrastructure, S. aureus, E. coli.

BACKGROUND

Nanotechnology has emerged recently in the material science field causing in short time a great impact in diverse contemporary fields as biology, chemistry and environment, energy, engineer, heavy industry and medicine[1,2]. The word ‘nano’ come from the Greek meaning ‘dwarf’ and was first applied in science in 1959 by Richard Feynman, a Nobel Prize winning physicist[3,4]. Nanotechnology can be defined as the science that involve the design, synthesis, characterization, and application of nanomaterials (all materials of size ranging between 1 nm and 100 nm in an unbound, or aggregate, or agglomerate state)[5,6].

Due to their small size, a collection of nanoparticles show a combined high surface area to volume ratio, resulting in unique physical, chemical, mechanical, electrical, optical and biological properties, which differ from their respective bulk material[7–10]. These distinct characteristics empower the interaction with cells and tissues at a molecular level in many ways, for example, as biological mimetics, nanomachines, carriers of drugs, gene therapy, and label; opening a new door at medical research: the nanomedicine[10].

Amongst the metallic nanoparticles, the most used in the biomedical field are gold nanoparticles (AuNPs)[11]. This preference prevails because gold is an easy material to obtain and manage; it is an inert metal; chemically stable; resistant to surface oxidation; non-toxic and compatible with cells[12,13]. Gold nanoparticles have

been already applied for the diagnosis and treatment of diseases, tissue/tumor imaging, drug delivery, photothermal therapy, antibody conjugation, and protection against UV rays[14,15].

AuNPs have emerged as potential alternative antibacterial agents against multidrug resistant (MDR) bacteria[16]. Since antibiotics became a medication with a broad and indiscriminate use, bacteria developed resistance against most standard antibacterial agents as well as adverse side effects due to higher dose prescription[17] resulting in a world health problem. Thus, the development of gold nanoparticles able to replace or improve those standard antibacterial agents may unveil novel biochemical and structural targets for the development of alternative therapies.

Nanoparticles are traditionally synthesized through chemical reduction of metal ions using toxic reducing agents to convert Au^{+3} ions to AuNPs[18,19]. However, these methods might represent an environmental and biological risk[20]. To overcome that limitation, new less expensive, chemically stable, environmentally-friendly, and clean biological methods using plant extracts as reduced and stabilizing agents have been recently developed[21]. Previous works have shown the application of green route synthesized AuNPs to control bacterial growth: AuNPs synthesized using *Ananas comosus* extract reduced the growth of *Staphylococcus aureus* (gram-positive) and *Pseudomonas aeruginosas* (gram-negative)[13]*;* also dextrose-encapsulated gold nanoparticles (dGNPs) showed significant antibacterial activity against both *Escherichia coli* (gram-negative) as well as *Staphylococcus epidermidis* (gram-positive) bacteria via disruption of cell membrane[21]. On the other hand, biosynthesized AuNPs with *Solanum nigrum* leaf extract had showed better antibacterial activity against gram-negatives bacterias than to gram-positives[22]. The anti-bacterial mechanism has electrostatic or mechanical interaction basis[13], with

direct dependence on composition, surface modification, intrinsic properties, and the bacterial species (cause of their different cell wall arrangement)[17].

Here, we investigated the potential antibacterial mechanism of gold nanoparticles reduced by *Virola oleifera* extract. *Virola oleifera* is a native plant of Atlantic forest being widely spread in the southeastern region of Brazil and has been applied in folk medicine as cicatrizant, anti-inflammatory, antirheumatic, and anti-asthmatic agents[23]. To verify if AuNPs biosynthesized with *Virola oleifera* produce ultrastructural changes in bacteria we tested the AuNPs with one gram positive (*Staphylococcus aureus)* and one gram negative (*Escherichia coli*) model bacteria and followed their growth along the time and their morphological changes by scanning and transmission electronic microscopy techniques.

METHODS

Green synthesis of gold nanoparticles

The gold nanoparticles have been synthesized by oxidation-reduction method, using a solution of $HAuCl_4$ (SigmaAldrich, St Louis, MO, USA) and solution of *Virola oleifera* resin at a concentration of 1000 µg/ml as the reducing agent. *Virola oleifera* was chosen because it is rich in phenolic compounds, which provides functionalization to the nanoparticles, and are important for reducing the metal ions, as well as have good antioxidant activity. In particular, 3 ml of solution of the *Virola oleifera* extract was added to 10 ml of $HAuCl_4$ (2.5×10^{-4} M) solution and shaken at 600 rpm for 10 minutes. The stirred solution was incubated at room temperature.

Antibacterial test

To measure the Minimum Inhibitory Concentration (MIC) of AuNPs against the

respective bacteria, the final solution of gold nanoparticles was diluted 10 and 100 times and inoculated with 1,5 x 10^6 UFC/mL in culture tubes containing sterile liquid media. Control experiments were performed by inoculating the media with bacteria and water. To ensure that the possible antimicrobial activity was due nanoparticles, a pure reducing solution was tested and at the same concentration used with nanoparticles. Two strains of bacteria were tested, the Gram positive *Staphylococcus aureus 1117* and the Gram-negative *Escherichia coli DH5α*. The 96-wells microplate was incubated with 100 µL of media plus bacteria with 100 µL of gold nanoparticles solution in their respective concentration at 37°C. The growth of bacteria was monitored by measuring the optical density (OD) at 620 nm for 24h using iMark microplate reader, BIO-RAD Lab. All the experiment was done in triplicate and the data were expressed by mean.

Scanning Electron Microscope analyse

To investigate if the gold nanoparticle from *Virola oleifera* produced any ultrastructural changes we compare the bacteria exposed to AuNPs by electron microscopy. The culture of bacteria strain was washed with PBS and fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4). After incubating with the fixative for 2 h at room temperature, samples were washed and centrifuged twice; the supernatant was discarded, and the pellet was resuspended in 0.1M sodium cacodylate buffer. The same process was followed during all subsequent solution changes. Samples were then post-fixed for 1 h at room temperature with 1% osmium tetroxide in 0.1M sodium cacodylate buffer. The post-fixed samples were washed with 0.1M sodium cacodylate buffer once and with distilled water twice and then dehydrated in a graded ethanol series (once in 30%, 50%, 70%, and 90%, and three

times in 100% ethanol for 10 min each). Following, the pellet was scattered using an ultrasonic cleaner (SB–100D) and 10 µl droplets applied on coverglass slide. The samples were critical point dried in a autosamdri-815 (tousimis) critical point dryer, attached to aluminum mounting stubs, sputter coated with gold-palladium, and imaged in a JEOL (JSM-6610LV, JEOL Inc., USA) scanning electron microscope.

Transmission Electron Microscope analyse

For TEM analysis, samples were prepared as described above for SEM with the difference in the dehydration with a graded acetone series (once in 30%, 50%, 70%, and 90%, and three times in 100% acetone for 10 min each). Following, the dehydrated samples were infiltrated with Epon's epoxy resin and acetone (1:1). Then left overnight in 100% resin. Samples were centrifuged through fresh resin in BEEM capsules (EMS Inc., Hatfield, PA, USA) and hardened at 60°C for 24h. Ultrathin sections (70nm) of the pelleted samples were cut on an ultramicrotome (RMC PowerTome X) using a diamond knife. The sections were stained with 2% aqueous uranyl acetate and Reynold's lead citrate for 5 minutes and 2 minutes, respectively for *S. aureus* and 20 minutes and 5 minutes, respectively for *E. coli* and examined using a transmission electron microscope (JEM-1400, JEOL Inc, USA).

Statistical Analyses

In order to measure the differences observed in structures between the control group and the group inoculated with AuNP a t-test analyse was performed using the GraphPad Prism 6 (GraphPad Software, La Jolla California USA). $p<0.05$ was taken as significant.

RESULTS

Synthesis of gold nanoparticles using Virola oleifera extract

The resulting solution showed a colour change from pale yellow to light pink. According to previous analyses with inductively coupled plasma mass spectrometry (ICP-MS) the final gold concentration was 40 µg/ml (data not shown). To use it in the antibacterial test this solution was diluted 10 times and 100 times resulting respectively in AuNPs of 4 µg/ml and 0.4 µg/ml gold concentration.

Antibacterial activity

The AuNPs were tested for their antibacterial activity at three concentrations against *S. aureus* and *E. coli*. In the 96-wells microplate, the bacterial cells grown in presence of AuNPs, and the growth was monitored hourly by measuring the OD at 620 nm for 24h. Optical density at 620 nm is a common method to quantify the concentration of bacterial cells in a liquid medium. There was no interference of AuNP or plant extract in the reading absorbance because they are read only at 530 nm and 300 nm respectively. Results were plotted with the OD on Y-axis against and the time on X-axis (Fig. 1). Growth curves of *Escherichia coli* did not show a significantly difference when compared to the control. At the end of 24 hours, the AuNPs showed inhibition only to *Staphylococcus aureus*. From the three concentrations used with *S. aureus,* the highest 40 µg/ml AuNP presents better performance keeping the bacterial population in lag phase for 8 hours after incubation while control stay in lag phase for only 4 hours. In addition, the *Virola oleifera* pure extract also showed higher inhibitory power. The 40 µg/ml AuNP follows the same behaviour than *Virola oleifera* pure extract (1000 µg/ml) suggesting the ability of the synthesized AuNPs to potentiate the antibacterial effects of this extract and allowing to obtain similar results using just a smaller concentration of extract.

An inhibition of approximately 65% on bacterial growth was observed to S. aureus (Fig 1).

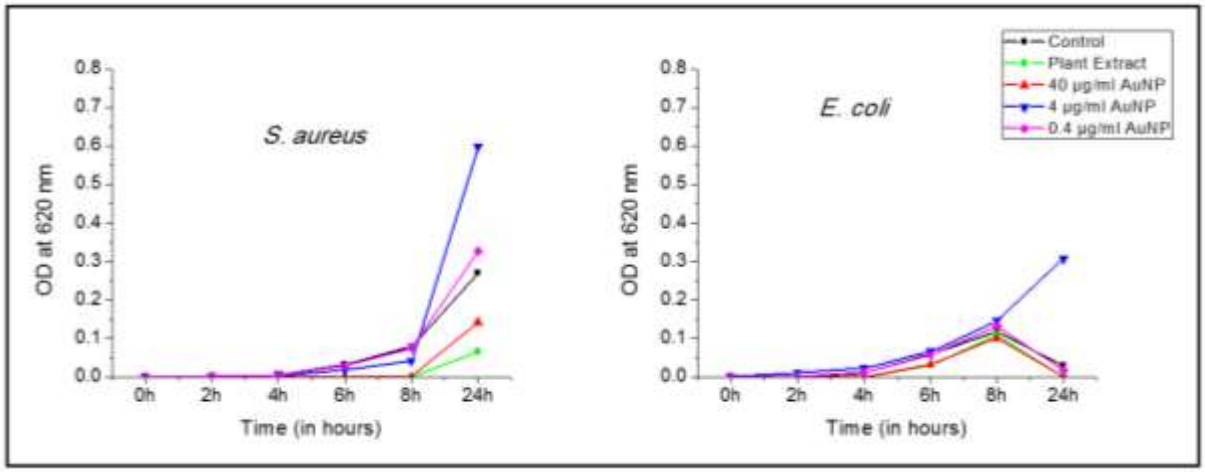


Figure 1. Effects of the AuNPs on the bacterial growth. Growth analysis curves were measured by monitoring the optical density (OD) at 620 nm, and the bacteria were treated with AuNPs at different concentrations (mg/L).

Scanning Electron Microscopy

Ultrastructural analyses were performed in *S. aureus* and *E.coli* treated with AuNP 40 mg/L due to its best results of antibacterial test presented above. The surface analysis by SEM indicate the presence of AuNPs around *S. aureus* cells and amorphous structures that resemble agglomerations of nanoparticles. Cells appear intact without any apparent damage (Fig. 2B). Few nanoparticles were observed interacting with *E. coli* cells (Fig. 2D). Fig. 2A and Fig. 2C show respective control samples.

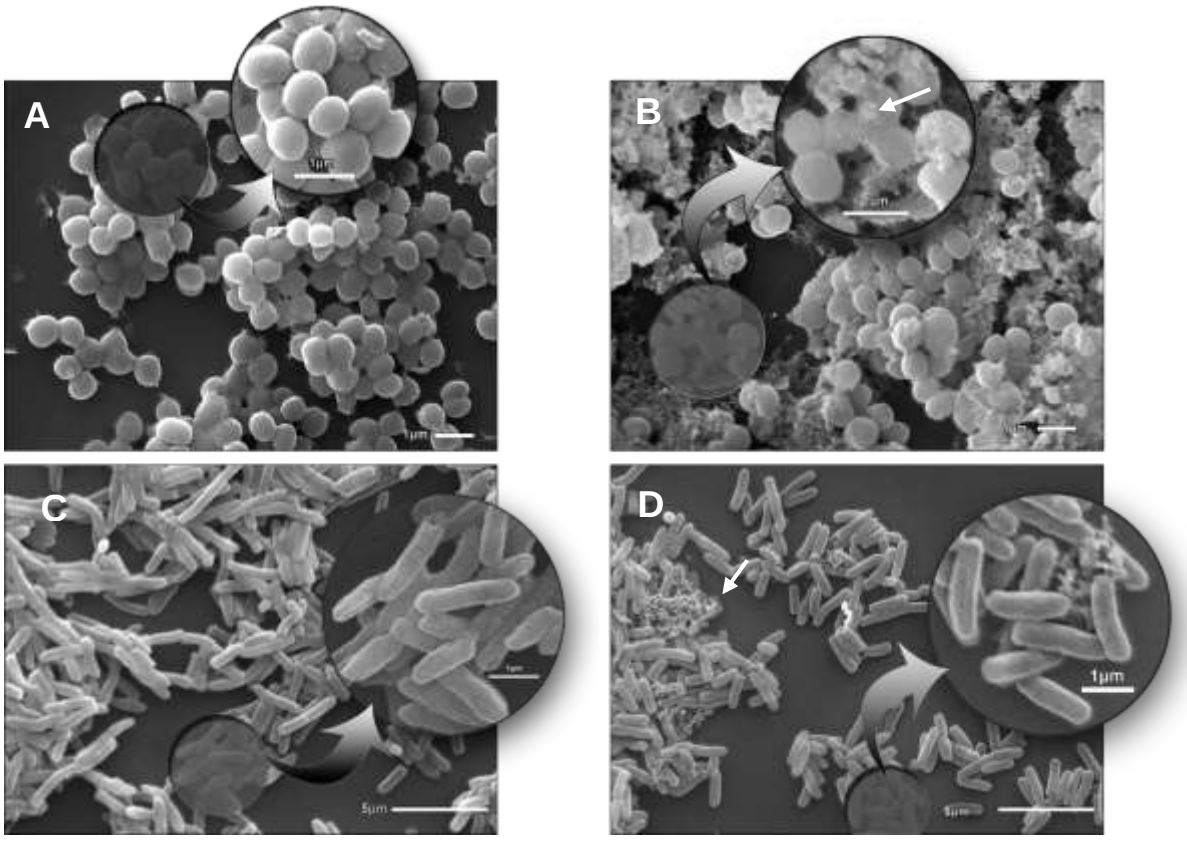


Figure 2. SEM micrographs of *S.aureus* and *E. coli*. **(A)** and **(C)** Control, **(B)** *S. aureus* treated with nanoparticles, **(D)** *E. coli* treated with nanoparticles. Arrows indicate AuNPs on contact with cells.

Transmission Electron Microscopy

E. coli treated with AuNPs do not show significant ultrastructural changes (Fig. 3) and it was hard to find gold nanoparticles interacting with gram negative bacteria. However, TEM micrographs confirmed the interaction and direct contact between *S. aureus* cells and AuNPs (Fig. 4).

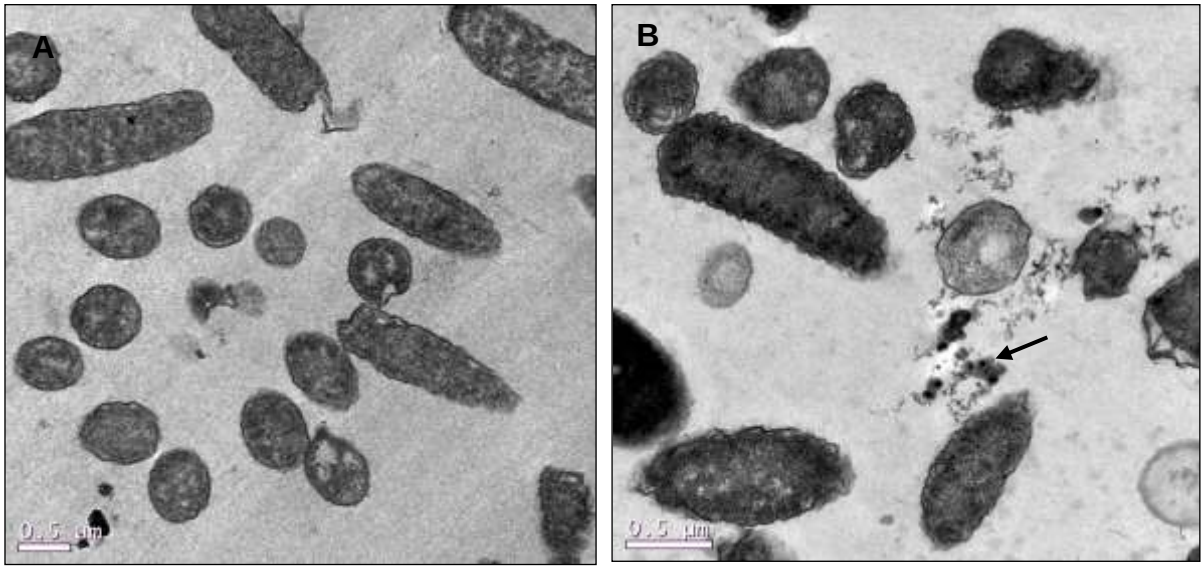


Figure 3. TEM micrographs of *E. coli*. No significative changes on structure the of cells were found. (A) Control, (B) few AuNPs were observed along the analyses. Arrow indicates AuNPs.

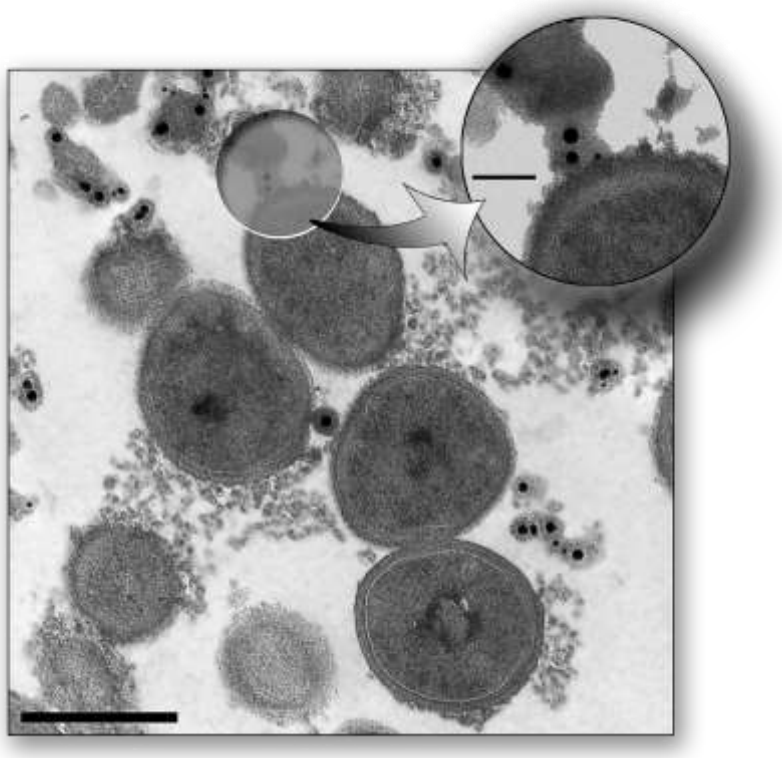


Figure 4. TEM micrographs of *S. aureus* in the presence of AuNPs. We can see in detail the nanoparticles in direct contact with the cell.

Furthermore, we observed injuries in the cell wall of *S. aureus* as well as increase on its thickness and modifications on membrane structure (Fig. 5).

To confirm the increase in cell wall thickness of *S. aureus* population exposed to AuNPs, the thickness of approximately 27 cells in the control sample and in the group treated with nanoparticles was measured to calculate the mean thickness of each group. Whereas the control group showed average of 25.7 nm, the group treated had average of 41.6 nm of cell wall thickness (64% increase) (Fig. 5D).

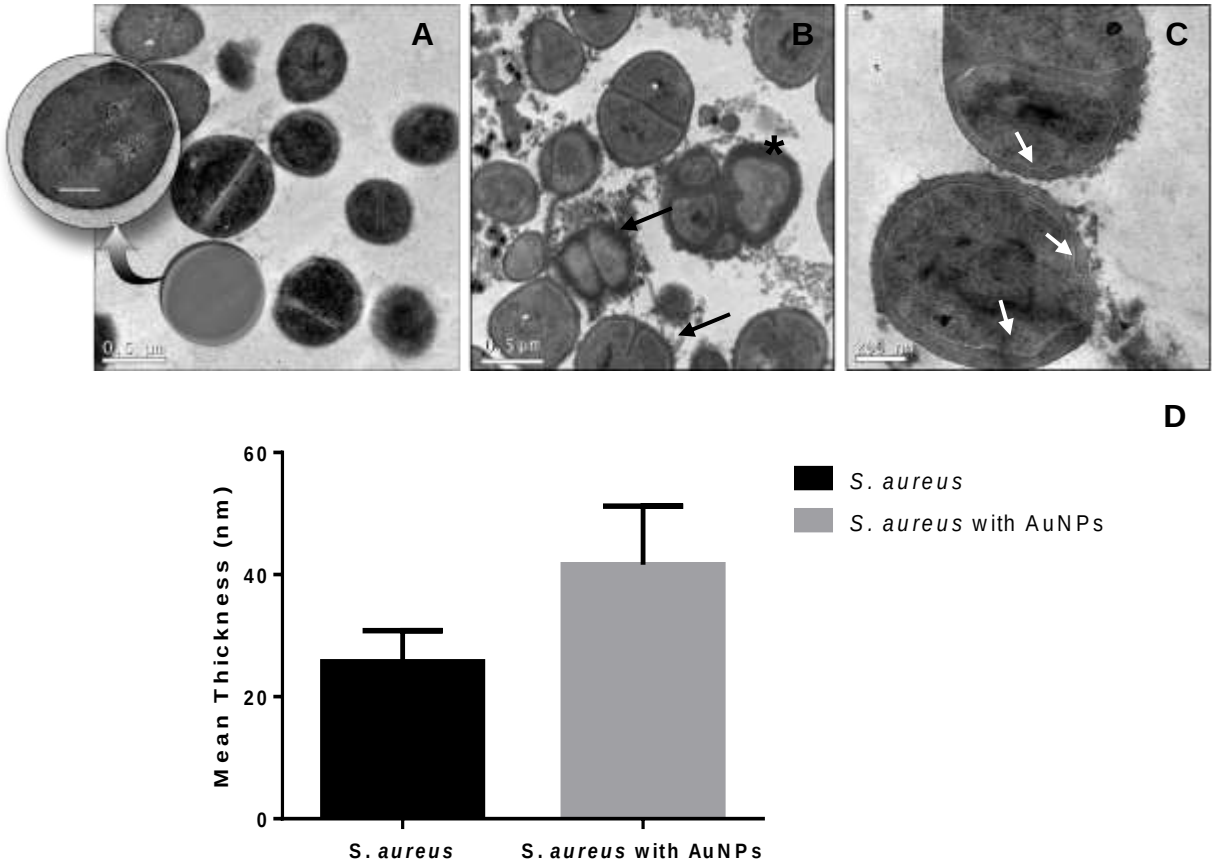


Figure 5. Ultrastructural changes induced by AuNP on *S. aureus*. **(A)** Control, **(B)** we can observe cell wall injuries (black arrows) and bacteria showing cell wall enlarged (*), **(C)** structural changes in the plasmatic membrane (white arrows), **(D)** graph comparing cell wall thickness. *S. aureus* treated with AuNP had a significative increase in cell wall compared with control ($p < 0.05$).

DISCUSSION

Antibacterial agents are commonly divided into two categories: as bactericidal, which kill bacteria; or bacteriostatic, which just slow down bacterial growth[17]. Throughout the data from antibacterial test, we observed that the gold nanoparticles synthesized with *Virola oleifera* extract as a reducing agent displayed an effective bacteriostatic action with effects only *S. aureus* growth. Comparing the antibacterial activity of the gold nanoparticles with the reducing agent (Fig. 1) it is observed similar antibacterial activity, meaning that even after reducing gold, the *Virola oleifera* resin functionalized effectively the nanoparticle, which conserve the high antibacterial power.

There are various potential mechanisms of gold nanoparticles against bacteria such as electrostatic interaction[24], disruption of cell membrane[25], induction of stress oxidative[26], and inhibiting ATP synthesis[27]; however the mechanisms of nanoparticles toxicity will depend not only of its composition, surface and properties but also of the bacterial species and morphology[20].

Thus, the bacteriostatic activity and ultrastructural changes here described against *S. aureus* but not *E. coli* are probably influenced by their cell wall composition. As known, *Staphylococcus aureus* is a Gram positive bacteria which means their wall contains a dense layer of peptidoglycan (PG) attached with teichoic acids that are unique to Gram positive bacteria[28]. On the other hand, Gram negative cell wall of *Escherichia coli* contains with a thin PG layer surrounded by an outer membrane rich in lipopolysaccharides which confers resistance to hydrophobic compounds and increase the negative charge of cell membranes[29]. Although both gram positive and gram negative bacteria have a negative membrane charges, the cell wall composition of *S. aureus* might modify its intensity and benefit the AuNPs

biosynthesized to attach on their cell wall.

A second fact to consider in the nanotoxicity analyses, it is the agglomeration of AuNPs as observed in Fig. 2B. Agglomeration could happen cause of difference of PH, or salts concentration between the AuNP's solution and the media where they were inoculated with bacteria. Agglomeration can change the size, surface area and sedimentation properties of the gold nanoparticles influencing their ability to reach the target and interact with bacteria[11,30].

Besides, growth rate has been reported to affect the bacteria resistance to antibiotics and nanoparticles. Species with fast-growing rates are more susceptible than slow-growing bacteria[31]. It is possible that the tolerance property of slow-growing bacteria is related to the expression of stress-response genes[32].

Distinctive of most nanoparticles that have been described, the AuNP synthesized from *Virola oleifera* extract does not cause inhibition through cell disruption and leakage[25,33,34,35]. Nevertheless, the modification of membrane structure, injuries and rise of thickness of cell wall visualised in *S. aureus* treated with AuNP (Fig. 5) may indicate an inhibition action by increasing the turgor pressure. Turgor pressure, or osmotic pressure, is created by the higher concentration of solutes in the cytoplasm and impulses the membrane against wall in gram positive bacteria[36].The cell wall is responsible for more than strength, rigidity and shape but is determinant to control the turgor pressure and protect from osmotic rupture[36,37]. Then, any modification on cell wall growth can disturb the turgor pressure. As the AuNPs touch the cell wall surface, it can breaks the peptidoglycan layer resulting in injuries. This could activate a defence mechanism, or even trick the cell, stimulating the wall's synthesis by laying down unstressed peptidoglycan in layers on the inner side of the wall while the outer layers are injuried[37,38] . It could increase the turgor

pressure causing stress on the membrane, inducing the expression of osmoregulation genes, influencing bacterial signal transduction systems, bacterial periplasmic transport functions, synthesis of porines, and finally inhibiting bacteria growth rate[39].

The results of this study demonstrated that the gold nanoparticle inhibited the growth and multiplication of the highly multidrug-resistant bacteria *S. aureus*. In spite of that antimicrobial potential, are necessary further studies to improve the nanoparticle behaviour and nanotoxicity using them as drug delivery mechanisms by conjugating with standard antibiotics[40,41].

In conclusion, the antibacterial activity of the green gold nanoparticle synthesized from *Virola oleifera* extract was confirmed against gram positive bacteria but not gram negative. We showed that AuNPs has bacteriostatic power against *S. aureus* changing the cell ultrastructure. The present study speculates a possible mechanism of growth inhibition based in the disturbing of turgor pressure and consequently internal homeostasis of cell. Thus, AuNPs represent a potential model for designing of antibacterial agents to target bacterial and to overcome drug resistance.

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