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Biosynthesis of silver nanoparticles by *Lentinus crinitus*: characterization and antimicrobial activity

Biossíntese de nanopartículas de prata por *Lentinus crinitus*: caracterização e atividade antimicrobiana

Biosíntesis de nanopartículas de plata por *Lentinus crinitus*: caracterización y actividad antimicrobiana

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Abstract
Silver nanoparticles (AgNP) obtained from biological synthesis can be widely used in industrial and medical fields because of their observed antimicrobial activity. The objective of this study was to analyze the biosynthesis of AgNPs by the fungus *Lentinus crinitus* (L.) Fr., and to evaluate the potential of these nanoparticles as antimicrobial agents. The antimicrobial activity of AgNPs was evaluated by agar diffusion, and broth microdilution methods to determine the minimum inhibitory concentration (CMI) against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Candida tropicalis*. AgNPs were characterized by UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), inductively coupled plasma emission spectrometry (ICP) and transmission electron microscopy (TEM). The UV-Vis spectra of the reaction mixture showed a SPR band with peak absorbance at 423 nm, confirming the presence of AgNPs. The synthesized AgNPs demonstrated antagonistic action against C. tropicalis (1.88 µg. mL^{-1}), C. albicans (30.09 µg. mL^{-1}), E. coli and *S. aureus* (7.52 µg. mL^{-1}). The AgNPs mediated by *L. crinitus* are mostly spherical, triangular and rod-shaped (mean diameter 8.82 nm). The concentration of silver in their crystalline structure is 120.37 µg / mL, and protein residues as possible stabilizers. The *Lentinus crinitus* mushroom isolated from substrates of the Amazon biome is a promising bio-resource for the biological synthesis of AgNPs with relevant antimicrobial properties and demonstrating a great potential for its application in pharmaceutical and food industries.
Keywords: Edible mushroom; Green synthesis; Silver nanoparticles; Cytotoxicity; Antimicrobial activity.

Resumo

As nanopartículas de prata (AgNP) obtidas a partir de síntese biológica podem ser amplamente utilizadas nas áreas industrial e médica devido à sua atividade antimicrobiana observada. O objetivo deste estudo foi analisar a biossíntese de AgNPs pelo fungo *Lentinus crinitus* (L.) Fr., e avaliar o potencial dessas nanopartículas como agentes antimicrobianos. A atividade antimicrobiana das AgNPs foi avaliada por métodos de difusão em ágar e microdiluição em caldo para determinar a concentração inibitória mínima (CMI) contra *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* e *Candida tropicalis*. As AgNPs foram caracterizadas por espectroscopia UV-Vis, difração de raios X (DRX), espectroscopia no infravermelho por transformada de Fourier (FTIR), espectrometria de emissão de plasma indutivamente acoplado (ICP) e microscopia eletrônica de transmissão (TEM). Os espectros UV-Vis da mistura reacional mostraram uma banda SPR com pico de absorbância em 423 nm, confirmando a presença de AgNPs. As AgNPs sintetizadas demonstraram ação antagônica contra *C. tropicalis* (1,88 µg. mL^{-1}), *C. albicans* (30,09 µg. mL^{-1}), *E. coli* e *S. aureus* (7,52 µg. mL^{-1}). As AgNPs mediadas por *L. crinitus* são em sua maioria esféricas, triangulares e em forma de bastonete (diâmetro médio de 8,82 nm). A concentração de prata em sua estrutura cristalina é de 120,37 µg/mL, tendo os resíduos proteicos como possíveis estabilizadores. O cogumelo *Lentinus crinitus* isolado de substratos do bioma Amazônia é um biorrecurso promissor para a síntese biológica de AgNPs com propriedades antimicrobianas relevantes, e demonstra grande potencial para sua aplicação nas indústrias farmacêutica e alimentícia.

Palavras-chave: Cogumelo comestível; Síntese verde; Nanopartículas de prata; Citotoxicidade; Atividade antimicrobiana.

Resumen

Las nanopartículas de plata (AgNP) obtenidas a partir de síntesis biológica pueden utilizarse ampliamente en los campos industrial y médico debido a su actividad antimicrobiana observada. El objetivo de este estudio fue analizar la biosíntesis de AgNPs por el hongo *Lentinus crinitus* (L.) Fr., y evaluar el potencial de estas nanopartículas como agentes antimicrobianos. La actividad antimicrobiana de las AgNP se evaluó mediante métodos de difusión en agar y microdilución en caldo para determinar la concentración inhibitoria mínima (CMI) contra *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* y *Candida tropicalis*. Las AgNP se caracterizaron mediante espectroscopia UV-Vis, difracción de rayos X (XRD), espectroscopia infrarroja transformada de Fourier (FTIR), espectrometría de emisión de plasma acoplado inductivamente (ICP) y microscopía electrónica de transmisión (TEM). Los espectros UV-Vis de la mezcla de reacción mostraron una banda SPR con un pico de absorbancia a 423 nm, lo que confirma la presencia de AgNP. Los AgNPs sintetizados demostraron acción antagónica contra *C. tropicalis* (1.88 µg. mL^{-1}), *C. albicans* (30.09 µg. mL^{-1}), *E. coli* y *S. a*ureus (7.52 µg. mL^{-1}). Los AgNP mediados por *L. crinitus* son en su mayoría esféricos, triangulares y con forma de bastón (diámetro medio 8,82 nm). La concentración de plata en su estructura cristalina es de 120,37 µg/mL, y residuos de proteínas como posibles estabilizadores. El hongo *Lentinus crinitus* aislado de sustratos del bioma amazónico es un biorrecurso prometedor para la síntesis biológica de AgNPs con propiedades antimicrobianas relevantes, y que demuestra un gran potencial para su aplicación en las industrias farmacéutica y alimentaria.

Palabras clave: Hongo comestible; Síntesis verde; Nanopartículas de plata; Citotoxicidad; Actividad antimicrobiana.

1. Introduction

Nanotechnology has gained considerable attention in the recent years due to the impact of nanostructured materials in the improvement of quality of lives of the people, and it represents a promising field in many sectors of the global economy, especially health, energy and agriculture, among others (Borase et al. 2014; Gurunathan et al. 2014; Khatoon, Ahmad, and Sardar, 2015). The global nanomaterial market is expected to grow by about 17% (Compound Annual Growth Rate) representing a profit of almost US $125 billion by 2024 (Research and Markets, 2018).

Among the nanomaterials, metallic nanoparticles (NMs) stand out because of their unique physical, chemical and biological properties, and are mainly used in sectors like electrochemistry, medicine, cosmetics, drugs and food (Niska et al. 2018). Silver nanoparticles (AgNPs) have several properties, presenting a wide spectrum of applications, and are widely recognized for their antimicrobial activities (Al-Thabaiti et al. 2015; Roy et al. 2019). Currently, AgNPs can be used in several areas such as food packaging (Simbine et al. 2019), textiles (Balamurugan, Saravanan, Soga, 2017), cosmetics (Naito et al. 2018) and health (Yesilot & Aydin, 2019).

The underlying mechanisms of the antagonistic activity of AgNPs against bacteria and fungi have not been fully understood. The antibacterial action of AgNPs seems to be related to morphological and structural changes that nanoparticles cause in bacterial cells due to their shape, ultra-small size and bigger surface area. These characteristics facilitate the penetration of the bacterial membrane causing intracellular damage (Rajeshkumar et al. 2019).

Several studies suggest that the antibacterial activity of AgNPs is due to the formation of reactive oxygen species, which cause changes in the structure of proteins and nucleic acids, and in the permeability of the cell wall, culminating in the lysis of the bacterial cell (Durán et al. 2016; Gudikandula et al. 2017; Flores-López et al. 2018; Liao et al. 2019).

Many techniques have been developed to synthesize AgNPs, including physical and chemical processes. However, these methods require equipments involving high operating costs, use of toxic chemical reagents, and generation of pollutant by-products (Bilal et al. 2017). The necessity to produce cost-effective eco-friendly AgNPs, which are also compatible to living systems, facilitated the emergence of biosynthesis, nanobiotechnology or green synthesis as an alternative to the existing techniques (Kulkarni & Muddapur, 2014; Ovais et al. 2017; El-Sayed et al. 2018; Gahlawat et al. 2019).

The biological synthesis of nanoparticles has been explored during recent years. Many biological resources, including plants, bacteria, filamentous fungi and mushrooms have been employed in the synthesis of nanoparticles (Keat et al. 2015; Barapatre et al. 2016; Devanesan et al. 2017; Khalil et al. 2017; Kumari, Barsainya, Singh, 2017; Mukherjee, Nethi, Patra, 2017; Nakkala et al. 2017; Khalil et al. 2018; Debnath, Das, Saha, 2019). This type of synthesis is considered low cost, sustainable, and can be performed under ambient temperature and pressure without using any external stabilizing agents. The biological synthesis of AgNPs by fungi is more efficient, as fungi produce large amounts of protein that contribute to high particle productivity and stability (Sanghi and Verma, 2009; Singh et al. 2014; Adeeyo et al. 2018; Avramescu et al. 2019). It is noteworthy that in biogenic synthesis, the reducing agent is also responsible for coating the surface of the obtained nanoparticles, increasing their stability and preventing their agglomeration (Zewde et al. 2016; Zhang et al. 2016).

Moreover, fungi can withstand harsh environments of bioreactors or chambers (Soni & Prakash, 2012), secrete large amounts of extracellular enzymes necessary for synthesis, and generate higher nanoparticles yield by mass (Alani, Moo-Young, Anderson, 2012; Vala et al. 2014).

There are few studies regarding the use of edible mushrooms in the synthesis of metallic nanoparticles. The use of *Lentinus crinitus* biomolecules to mediate the synthesis of AgNPs may add interesting properties to the nanomolecules, which may increase the antimicrobial action, considering that this species already produces antagonistic substances for other microorganisms (Li et al. 2011; Iravani, Thota, Crans, 2018).

Considering the importance of using silver nanoparticles (AgNPs) obtained from mushrooms of the Amazon biome, the objective of this study was to analyze the biosynthesis of AgNPs through the biochemical reduction of silver ions, mediated by the use of the fungus *Lentinus crinitus* (L.) Fr., and to evaluate the potential of these nanoparticles as antimicrobial agents.

2. Methodology

2.1 Reactivation of culture

The lineage *Lentinus crinitus* (L.) Fr. 1825 DPUA 1693 used in this research was provided by the DPUA Culture Collection of the Federal University of Amazonas. The culture was preserved in mineral oil and was reactivated in glycoside broth (1% meat peptone, 0.3% yeast extract and 2% glucose) in a stationary culture for 15 days. Subsequently, subculture was carried out on potato dextrose agar (PDA) medium, added with 0.5% yeast extract (w/v) using 8 mm mycelial as inoculum at the center. The cultivation was maintained at 25 ºC, and in the absence of light for eight days (Kirsch et al. 2011).

2.2 Biomass Production in Submerged Cultivation

For the production of biomass, the fungus was grown in 200 mL of GYP medium (2% glucose; 0.5% yeast extract and 0.5% peptone [w / v]) in a shaker (150 rpm) at 30 ºC for 120 h, using ten mycelial discs (Ø = 8 mm) as inoculum. The biomass was separated by filtration using Whatman filter paper n.1 and washed three times with ultrapure water to remove residues of the medium. The mycelial mass was placed in an Erlemneyer flask containing 200 mL of ultrapure water and the biomolecules were extracted for 120 h, at 30 ºC, at 150 rpm. The biomass was filtered, and the aqueous extract was recovered and used for biosynthesis of silver nanoparticles (Durán et al. 2007; Silva et al. 2017).

2.3 Synthesis of nanoparticles using aqueous extract

The biosynthesis of AgNPs occurred from the reaction of 200 mL of the aqueous extract with silver nitrate solution (1 mol. L^{-1}) until the final concentration of 1 mmol. L^{-1} of $AgNO_3$ was obtained. The reaction was carried out in the dark at a temperature of 28 ºC, at 150 rpm for 5 days, after which the reaction mixture was left at room temperature without stirring until the surface plasmon resonance (SPR) band was stabilized. The cell filtrate (without silver nitrate) and a 1 mmol. L^{-1} solution of $AgNO_3$ were used as controls. The synthesis of silver nanoparticles was observed by the color change of the solution, and later confirmed by the presence of plasmon resonance band in the region of 400 nm (Zomorodian et al. 2016). The measurements of the absorption spectra were carried out with a resolution of 1 nm in a reading range of 200-800 nm using UV-Vis spectrophotometer (Cary 60, Agilent Technologies).

2.4 Characterization of silver nanoparticles

2.4.1 X-Ray diffraction (XRD)

The crystalline nature of the lyophilized AgNPs was determined by diffractometry at the Mineralogical Techniques Laboratory of the Geology Department in the Federal University of Amazonas (UFAM), using Shimadzu's X-ray diffraction apparatus (model XRD6000- operating with CuKα radiation) with the following parameters: 40kV voltage, 30mA current, and a scanning speed of 2° min^{-1} with an angle of 2θ between 20 and 90°.

2.4.2 *Fourier* Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) was performed at the Biodegradation Laboratory of the Federal University of Amazonas (UFAM), using the Iraffinity-1 Shimadzu spectrophotometer, in between the length range of 700 to 4000 cm^{-1}, with a resolution of 7 cm^{-1}. Potassium bromide (KBr) and 1 mg of AgNPs were compacted in a hydraulic press until a tablet form was obtained for analysis in the device.

2.4.3 Inductively coupled plasma emission spectrometry (ICP)

The quantification of the total amount of silver (Ag) present in the AgNP solutions was carried out in a THERMO ICP-OES spectrophotometer, model ICAP-7600, at the Laboratory of Environmental Analytical Chemistry (LQAA), Coordination of Environmental Dynamics (CODAM) in the National Institute of Amazonian Research (INPA). The calibration curve for the analyzed element (Ag) was constructed from nine silver nitrate solutions with concentrations ranging from 0.1 to 50 mg/L. The AgNPs solution was diluted 10 times to facilitate reading on the equipment.

2.4.4 Energy dispersion X-ray spectroscopy (EDS)

The analysis of X-ray spectroscopy by energy dispersion was carried out at the Multi-User Center for Analysis of Biomedical Phenomena (CMABio) at the State University of Amazonas (UEA). The data were obtained using a Jeol JSM-

IT500HR scanning electron microscope coupled to an X-ray spectrophotometer by energy dispersion using 10 kV electron acceleration voltage and 40,000x magnification. The sample of lyophilized AgNPs was placed under a support (*stub*) covered with a carbon ribbon for these analyses.

2.4.5 Transmission Electron Microscopy (TEM)

The morphological characterization of AgNPs biosynthesized by *Lentinus crinitus* was carried out at the Centre of Instrumental Chemical Analysis (CAQI), Institute of Chemistry of São Carlos (IQSC) of the University of São Paulo (USP), from high resolution images in Transmission Electron Microscope - TEM (JEM-2100-JEOL LaB6 equipment) operating at an acceleration voltage of 200 KeV. To obtain the images, a drop of the nanoparticle solution (diluted 50 times) was placed on a carbon-coated copper grid (400 mesh) and dried at room temperature. The diameter of the AgNPs was calculated in the software *image J*, and the statistical analysis for histogram was performed with *origin* (version 8.5.1.315).

2.5 Antimicrobial activity of AgNPs in Solid Media

The antimicrobial activity of the nanoparticles was performed in the Collection of DPUA Culture of the Federal University of Amazonas against four test microorganisms: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* CBAM 001, *Candida albicans* DPUA 1706 and *Candida tropicalis* DPUA 023 by agar perforation diffusion technique (Silva et al. 2017). The tests with bacteria were performed on Müller-Hinton Agar media (inoculum containing 108 CFU / mL) and the yeasts on Sabouraud Agar media (inoculum containing 106 CFU /mL), in Petri dishes, at 37 ° C, in triplicate. Cell suspensions (100 µL) were seeded with the aid of a Drigaslki loop on the surface of the culture media. In each well (Ø = 8 mm), 100 µL of the positive control (itraconazole solutions 50 µg/mL for yeast and streptomycin 50 µg / mL for bacteria) and the suspension of AgNPs were added. The plates were incubated at 37°C for 24h (bacteria) and 48h (yeast). Antimicrobial activity was expressed in millimeters, by measuring the diameter of the inhibition zones (Prado et al. 2017).

2.6 Minimum Inhibitory Concentration (MIC) of Silver Nanoparticles

The Minimum Inhibitory Concentration (MIC) was determined based on the broth microdilution methodology (M7-A6 and M27-A2 of the CLSI [Clinical and Laboratory Standards Institute, 2008)], against the test microorganisms: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* CBAM 001, *Candida albicans* DPUA 1706 and *Candida tropicalis* DPUA 023. The experiment was carried out in 96-well microplates, where the bacteria (5 x 105 CFU/mL) and yeast (2 x 103 CFU/mL) were exposed to diluted nanoparticles [(0.11 to 60.18 µg/mL)]. As positive controls, solutions of itraconazole and streptomycin (200 µg/mL) were used. The bacteria and yeasts were incubated at 37ºC, for 24h and 36h, respectively. After that period, 10 µL of resazurin (200 µg/mL) was added to each well and a new incubation was performed at 37°C for two hours to show the growth of microorganisms. The assays were performed in triplicate and the MIC was defined to be the lowest concentration of the agent in µg/mL where inhibition of the microorganism was observed (Prado et al. 2017).

3. Results and Discussion

3.1 Surface plasmon resonance and analysis of UV-Vis spectra

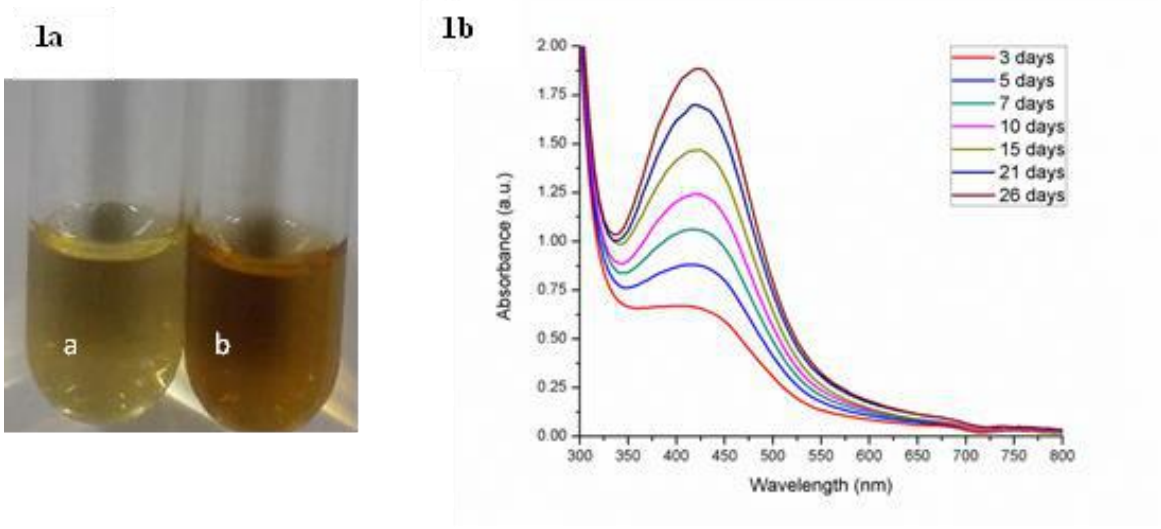
The synthesis of AgNPs mediated by *Lentinus crinitus* was observed in the chromatic alteration of the reaction substrate, as the color change is a result of the biotransformation of Ag^{+} ion into Ag^{0} (Figure 1A), indicating the synthesis of silver nanoparticles (Li et al. 2012; Jena et al. 2013). The color change is related to the effect of surface plasmon resonance (SPR), a phenomenon associated with absorption in the ultraviolet regions, and visible in the electromagnetic spectrum, which

occurs by the collective oscillation of surface electrons of metal after the incidence of electromagnetic radiation (Thukkaram et al. 2014; Kumar, Kumar, Agrawal, 2018).

Figure 1B shows the UV-Vis spectra of biosynthesized AgNPs as a function of time intervals. The formation of AgNPs is expressed by the different colored absorption peaks at different time intervals (3, 5, 7, 10, 15, 21, 26 days). After 26 days, no change in the spectrum was observed, which indicated the complete consumption of $AgNO_3$ precursor in the reaction mixture. The SPR band obtained at the end of the reaction displayed a peak at 423 nm, indicating the synthesis of silver nanoparticles by the extract of *Lentinus crinitus*. Similar results have been observed in several studies with synthesis of silver nanoparticles in mushrooms. In their study with the extract of *Pleurotus florida* mushroom, Kaur, Kapoor and Kalia (2018) obtained SPR peaks around 400 to 450 nm at different time intervals. Mohanta (2018) used wild mushroom *Ganoderma sessiliforme* and obtained a characteristic peak of 432 nm. Debnath, Das and Saha (2019) used extracts of wild edible mushrooms *Pleurotus giganteus* and obtained a peak of 420 nm. Abikoye et al (2019) used the extract of the edible mushroom *Nigeria Auricularia polytricha*, and also observed a peak of 420 nm.

There are several studies that prove that the peak of the plasmatic resonance band for AgNPs is located within a spectral range of 400 and 530 nm (Lee & El-Sayed, 2006; Ghorbani & Safekordi, 2011; Firdhouse & Lalitha, 2015; Abbasi et al. 2016; Deljou & Goudarzi, 2016).

Figure 1 – (1a) Color change for the reaction of synthesis of silver nanoparticles: aqueous extract from the biomass of *Lentinus crinitus* (a) without $AgNO_3$ (b) with $AgNO_3$ (1 mol. L^{-1}); (1b) UV-vis absorption spectrum of *Lentinus crinitus* AgNPs at different time intervals.



Source: Authors.

The color change is a result of the biotransformation of Ag^+ ion into Ag^0 (Figure 1a), indicating the synthesis of silver nanoparticles. Figure 1b shows the UV-Vis spectra of biosynthesized AgNPs as a function of time intervals. After 26 days, no change in the spectrum was observed, which indicated the complete consumption of $AgNO_3$ precursor in the reaction mixture.

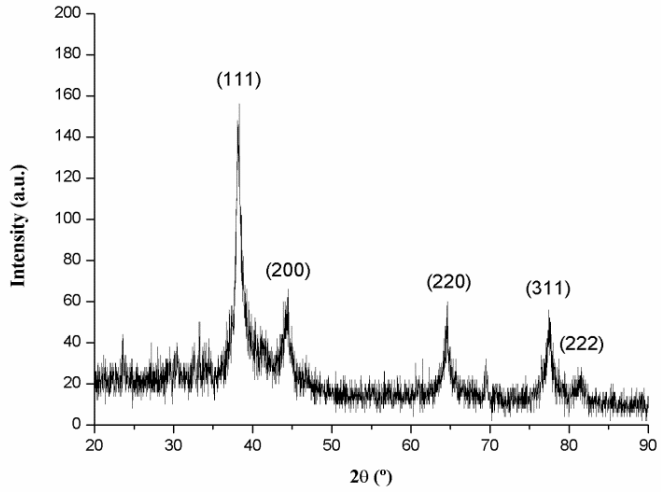
3.2 Characterization of AgNPs

3.2.1 X-Ray diffraction analysis

The crystalline nature of silver nanoparticles can be determined by X-ray diffraction (XRD) spectrum of the samples. Figure 2 shows the XRD pattern of AgNPs synthesized from the aqueous extract of *the Lentinus crinitus* biomass. The XRD

peaks at 2θ degrees appear at 38.28°, 44.38°, 64.60°, 55.24° and 81.52°; this can be attributed to the (111) (200) (220) (311) and (222) crystalline planes of the face centered cubic crystalline structure of metallic silver (Roy, Sarkar, Ghosh, 2015; Dauthal & Mukhopadhyay, 2016; Sarwar et al. 2018; Rolim et al. 2019). The presence of these five crystalline planes confirms the formation of metallic silver in the suspension of AgNPs (Mukherjee, et al. 2008). The sharpness of the peaks indicates that the particles are at nano scale (Bar et al. 2009; Bankar et al. 2010). The intensity of silver nanoparticles is an indication of a high degree of crystallinity according to the findings of other researchers (Dada et al. 2018a, 2018b; Chen et al. 2017; Jassal et al. 2016).

Figure 2 – Diffractogram of lyophilized AgNPs biosynthesized by aqueous extract of *Lentinus crinitus*.



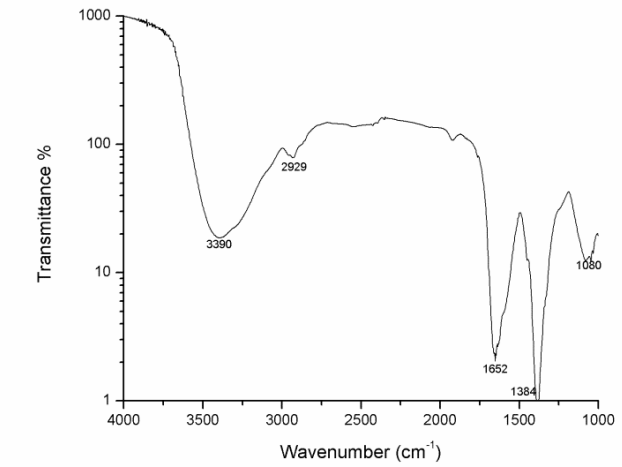
Source: Authors.

Figure 2 shows the XRD pattern of AgNPs synthesized from the aqueous extract of *the Lentinus crinitus* biomass. The presence of these five crystalline planes confirms the formation of metallic silver in the suspension of AgNPs.

3.2.2 *Fourier* Transform Infrared Spectroscopy (FTIR) analysis

The FTIR spectrum enables the identification of functional groups linked to the surface of metallic nanoparticles (Acay & Baran, 2019). The FTIR analysis spectrum of the colloidal AgNPs solution synthesized by *Lentinus crinitus* resulted in five distinct peaks: 3390, 2929, 1652, 1384 and 1080 cm^{-1} (Figure 3). Peak 3390 corresponds to the stretching of -OH group (Bhatnagar et al. 2019), peak 2929 represents the stretching of the C–H bond (Al-Hamadani & Kareem, 2017), peak 1652 corresponds to the stretching bond of the C=O group present in tertiary amides (Biswas & Mulaba-Bafubiandi, 2016), and peak 1384 is attributed to the stretching of the C–N bond present in aromatic amines (Biswas & Mulaba-Bafubiandi, 2016). The peaks located between 1384 cm^{-1} and 1080 cm^{-1} indicate the C-N stretching vibrations of aromatic and aliphatic amines, respectively (Biswas & Mulaba-Bafubiandi, 2016). FTIR data demonstrated that the presence of carbonyl and amine groups may indicate the existence of a protein cover around AgNPs, which facilitates the stabilization of nanoparticles in the medium (Shanmuganathan et al. 2018; Thokala et al. 2018; Omran et al. 2018).

Figure 3 – FTIR spectrum of *Lentinus crinitus* AgNPs.



Source: Authors.

The FTIR spectrum enables the identification of functional groups linked to the surface of metallic nanoparticles. FTIR data demonstrated that the presence of carbonyl and amine groups may indicate the existence of a protein cover around AgNPs, which facilitates the stabilization of nanoparticles in the medium.

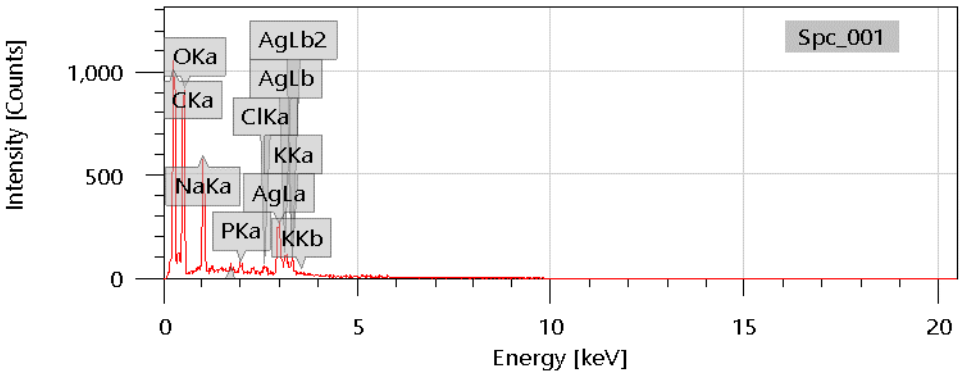
3.2.3 Inductively coupled plasma emission spectrometry (ICP)

Inductively coupled plasma emission spectrometry analyses determined that the silver concentration in the colloidal suspension of AgNPs obtained after its biosynthesis by *Lentinus crinitus* was 120.37 µg/mL. In their study with the fungus *Aspergillus oryzae*, Silva et al. (2017) obtained a similar concentration of silver in AgNPs suspension (134.5 µg/mL).

3.2.4 Energy dispersion X-ray spectroscopy (EDS)

The EDS spectra revealed three peaks close to 3 KeV (Figure 4), corresponding to the energy that make up the optical absorption profile of the silver element and its isotopes (AgLa and AgLb and AgLb2) (Pereira et al. 2014; Elgorban et al. 2016).

Figure 4 – Energy dispersion X-ray spectrum (EDX) of AgNPs biosynthesized by the aqueous extract of the *Lentinus crinitus* biomass.



Source: Authors.

The EDX spectrum reveals the purity and the complete chemical composition of AgNPs.

In addition to silver, the spectrum generated by EDS detected the presence of carbon (C), oxygen (O), sodium (Na), silicon (Si), phosphorus (P) and chlorine (Cl), the elements that are part of natural composition of cells and may be associated with proteins. The EDS analysis also revealed that silver is the second most abundant element in the suspension of AgNPs with 29.41% of mass, surpassed only by carbon with 30.61% of mass, a result which can be justified by the presence of biological material extracted from the mycelium of *L. crinitus* (Table 1).

Table 1 – Elementary composition of AgNPs in X-ray energy spectrum (EDX).

Microrganism	Minimum inhibitory concentration (µg.mL^{-1})
Candida tropicalis DPUA 023	1.88 ± 0,10
Candida albicans DPUA 1706	30.09 ± 0,20
Staphylococcus aureus ATCC 25923	7.52 ± 0,20
Escherichia coli CBAM 001	7.52 ± 0,30

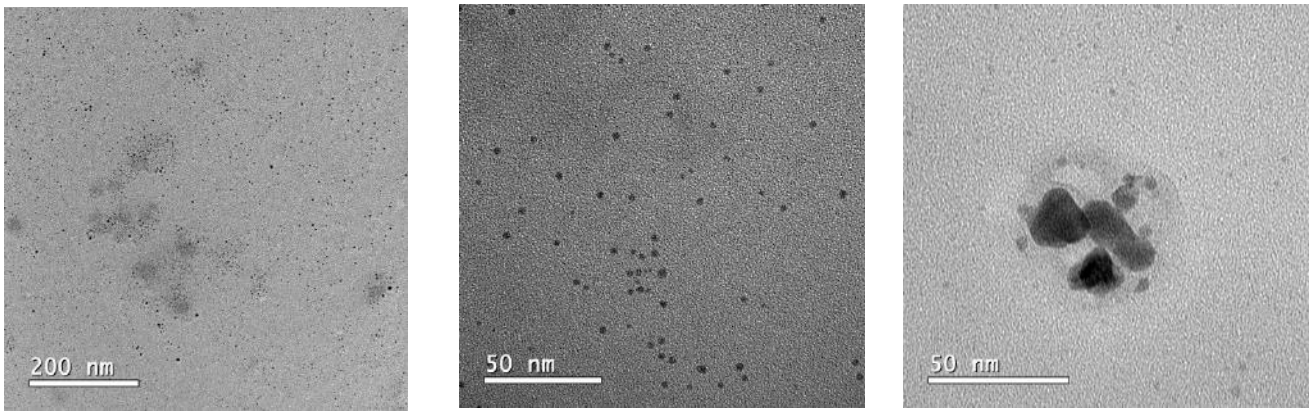
Source: Authors.

Table 1 shows the data referring to the minimum inhibitory concentration of AgNPs colloidal solutions. The colloidal solution of AgNPs from Lentinus crinitus showed the same MIC for *E. coli* and S. aureus, with values of 7.52 µg/mL, 30.09 µg/mL for *Candida albicans* and 1.88 µg/mL for *Candida tropicalis*.

3.2.5 Transmission Electron Microscopy (TEM)

The TEM image of the AgNPs from the *Lentinus crinitus* mushroom revealed that most of the AgNPs are spherical, however some were triangular, square and rod shaped (Figure 5). The average diameter of these nanoparticles is 8.82 nm, ranging from 1-92 nm. The size distribution histogram of the nanoparticles shows that most of them are within the range of 0 and 10 nm. Kaur, Kalia and Sodhi (2020), in their study with *Pleurotus florida* mushrooms, obtained TEM images of spherical nanoparticles with sizes ranging from 5-40 nm. Debnath, Das and Saha (2019), in their study with *Pleurotus giganteus* mushrooms, obtained spherical AgNPs with sizes between 5 and 25 nm. In their study with *Auricularia polytricha* mushroom, Abikoye et. al. (2019) found spherical AgNPs with sizes between 5 and 50 nm.

Figure 5 – Transmission electron microscopy (TEM) image of AgNPs biosynthesized by the aqueous extract of *Lentinus crinitus*.



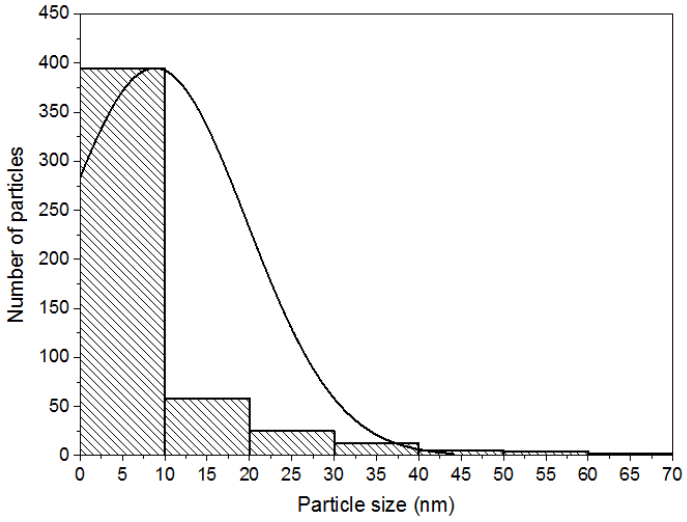
Source: Authors.

Transmission electron microscopy (TEM) imaging reveals the size, shape and general morphology of nanoparticles. It also confirms that most of the formation of AgNPs from the *Lentinus crinitus* mushroom were spherical.

3.3 Antimicrobial activity of AgNPs in Solid Media

The AgNPs synthesized by *Lentinus crinitus* in the silver concentration of 120.37 µg/mL showed antimicrobial activity in the agar diffusion method, against *S. aureus*, *E. coli* and *Candida tropicalis* while *C. albicans* was resistant for the tested concentration. The greatest inhibition was observed against *S. aureus* (17.7 ± 0.2 mm), followed by *E. coli* (14.3 ± 0.3 mm) and *Candida tropicalis* (12.0 ± 0.1 mm) (Figure 6).

Figure 6 – Histogram of size distribution of AgNPs biosynthesized by aqueous extract of *Lentinus crinitus*.



Source: Authors.

The small average size of the AgNPs has a role to play in its antimicrobial activity. Thus, the smaller the nanoparticles, the greater the antimicrobial activity.

Silver nanoparticles can be used effectively against multi-resistant bacteria due to their small size, shape and large surface area that provides greater contact with microorganisms, moreover, bacteria do not develop resistance to silver (Evans & Markose, 2014; Wang et al. 2017; Nakamura et al. 2019).

Guilger-Casagrande and Lima (2019) reported that AgNPs are being used in association with antibiotics and antifungals, thereby providing an alternative approach for the inhibition of multiresistant bacterial growth.

3.4 Minimum Inhibitory Concentration (MIC) of Silver Nanoparticles

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that inhibits the growth of microorganism under standardized conditions (Oliveira et al. 2009). *L. crinitus* AgNPs were more efficient to inhibit the growth of *C. tropicalis*, with an MIC value of 1.88 µg. mL^{-1}, while for *C. albicans* it was 30.09 µg. mL^{-1} (Table 2). Similar results were observed by Rodrigues et al. (2012) in their study of AgNPs synthesized by filamentous fungi, in which the MIC value for *C. tropicalis* was 0.44 µg. mL^{-1} and for *C. albicans* it was 27.30 µg. mL^{-1}.

Table 2 – Minimum inhibitory concentration (MIC) of AgNPs by *Lentinus crinitus* on different test microorganisms.

Microrganism	Minimum inhibitory concentration (µg.mL^{-1})
Candida tropicalis DPUA 023	1.88 ± 0,10
Candida albicans DPUA 1706	30.09 ± 0,20
Staphylococcus aureus ATCC 25923	7.52 ± 0,20
Escherichia coli CBAM 001	7.52 ± 0,30

Source: Authors.

Table 2 shows the antimicrobial activity of lentinus silver nanoparticles (AgNPs) against different pathogenic microorganisms at various concentrations investigated.

AgNPs interact with yeast cells causing changes in the permeability of cell membrane and derangement of the lipid bilayer, resulting in the escape of ions and other materials, as well as formation of pores that dissipate the electrical potential of the membrane and cause cell death (Kim et al. 2009 & Silva et al. 2017).

The tested bacteria were sensitive to the MIC of 7.52 µg. mL^{-1}. In their study of AgNPs synthesized by *Pleurotus giganteus* mushrooms, Debnath, Das and Saha (2019) found the MIC for *S. aureus* to be 9.16 µg. mL^{-1}, and for *E. coli* to be 11.6 µg. mL^{-1}. Although AgNPs' antimicrobial activity has been extensively proven, the mechanisms of action of these particles on bacteria and fungi are not yet fully understood. Several studies suggest that the antibacterial activity of AgNPs is due to the generation of reactive oxygen species (ROS), which cause changes in the structure of proteins and nucleic acids, and affects the permeability of the cellular membrane, culminating in the lysis of the bacterial cell (Figure 5) (Dakal et al. 2016; Gudikandula et al. 2017; Siddiqi, Husen, Rao, 2018).

4. Conclusion

The present research revealed that the mushroom *Lentinus crinitus* is a promising species for biosynthesis of silver nanoparticles. The AgNPs synthesized by *L. crinitus* had a spherical shape, nanometric size, crystalline nature and anti-microbial activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida tropicalis* and *Candida albicans*. This result signifies a safe alternative approach for the production of new antimicrobial agents with AgNPs, since the molecules used for the synthesis and stabilization of the nanoparticles come from edible mushrooms. In addition, the AgNPs synthesized by *L. crinitus* is derived from an eco-friendly process, so the resulting product with antimicrobial activity will have great potential to be used in diverse areas of health and food industries.

The biotechnological application of the *Lentinus crinitus* mushroom in the synthesis of silver nanoparticles represents a viable alternative for the elaboration of nanostructured products that can be useful in the areas of food, pharmaceuticals and medicine. Knowledge of the potential of this species can contribute to the rational use of Brazilian biodiversity, allowing the wealth of our forests to be turned into sustainable economic development.

The literature on this mushroom is very scarce, so the dissemination of information about the activities of this fungus can serve as a stimulus for new research with mushrooms from the Amazon. *Lentinus crinitus* AgNP can be recommended as a valuable product in the field of nanobiotechnology and nanomedicine.

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