Covenant Journal of Physical & Life Sciences (CJPL) Vol. 7 No. 1, March, 2019 (SE)

An Open Access Journal Available Online

Biosynthesis of silver nanoparticles in improved strain of Auricularia polytricha -an edible mushroom from Nigeria and its antimicrobial activities.

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Received: 21st September, 2018; Accepted: 7th February, 2019;
Online Published: May 7th, 2019.
URL http//Journal.covenantuniversity.edu.ng/cjoe/

Abstract: Nano materials and their application are of great use in research because of their size range. In this study, Auricularia polytricha (EW1) collected from Benin in Southern Nigeria was subjected to mutation by exposure to UV-light resulting in a mutant (EW1M1). EW1 and EW1M1 were evaluated for biosynthesis of silver nano-particles. The anti-microbial properties of both strains were also evaluated. Absorption spectra of silver nano-particle (AgNPs) of mutant exhibited a strong broad peak at 420 nm while wild type absorption peak was obtained at less than 420 nm. The mutant was further characterised. DLS showed a monodispersion with diverse sizes, morphology and shapes. TEM micrograph revealed a monodispersed formation of the nano-particles, with uniform size at 10 – 20 nm. FTIR study revealed the absorption bands at 3380, 2921, 2839, 1658, 1083 and 610 cm¯1 respectively showing the functional groups reducing the silver nitrate to silver ion. There was formation of zone of inhibition on all the microorganisms that were used for the study but the control showed no zone of inhibition. The mushroom extract of mutant strain exhibited higher anti microbial activity than the wild type.

Keywords: Auricularia polytricha, Silver Nano-particle (AgNPs), UV- Vis Spectra, Antimicrobial Activities.

Introduction

Auricularia species is a soft, jelly like edible mushroom. It is hunted and consumed by the Benin people in Southern area of Nigeria [1]. In Ghana, the mushroom is used as blood tonic [4]. It is a popular ingredient in many Chinese dishes, and medicines. Medically, the

polysaccharides extracted from the mushroom, help stimulate the immune system in humans, because the production of interferon and interleukins that stop the proliferation of cancer cells [17]. Other medical properties of Auricularia species include its antitumor, hypoglycemic, anticoagulant and cholesterol lowering properties [5]. Mushrooms are good antioxidants [6]. It also possesses antiviral and anti-cancer properties [20].

Nanotechnology is concerned with the synthesis of nanoparticles of variable sizes, shapes, chemical composition and controlled disparity. Nanoparticles are particulate or dispersions of solid particles with size range of 10 to 1000 nm [18]. They possess physical, chemical, electronic/electricalmechanical, thermal, dielectric, optical and biological properties that are different from the bulk material of the same element [18]. The optical, physicochemical and electronic properties of nanoparticles vary with difference in their size, shape and crystalline formation. The field of nanotechnology has gained great importance because of its potential applications in various fields such as chemical and textile industries, medicine/drug gene delivery and computer etc.

Silver nanoparticles are known to possess strong antimicrobial properties and that is one of the major reasons for the development of nanosilver containing products. Out of over 1000 consumer products that contain nanomaterials, about 25 % are believed to contain silver nanoparticles. Some of the consumer products that contain nanosilver particles include food contact materials (such as cups, bowls, cutting boards, etc.), odour resistance textiles, electronics and other household appliances [8, 19] The AgNPs has been reported to have antimicrobial activity against human pathogens [12] and it has been used to solve many problems which are related to harmful and toxic by-products from artificially made products [11]. The use of AgNPs helps as resistance by some pathogenic microorganisms in multidrugs is eliminated because of their small size over their large surface area which increases their antibacterial efficiency [18, 19]. Thus synthesising nanotechnology in cosmetics, ATM buttons, medical, household appliances, baby toys, sport equipment and clothing the products to have antibacterial properties [3, 11, 8]. The silver nanoparticle process is biosafe, harmless to the surrounding cells of the body and eco-friendly over conventional antibiotics because they help destroy all pathogenic microorganisms which no organism has been reported to develop resistance to [2, 19]. In this work, we have used extract of edible Nigeria mushroom Auricularia polytricha which is also known as Wood ear mushroom for the synthesis of bio functional silver nanoparticles.

II Materials and Methods

Sample collection

Fresh sample of Auricularia polytricha growing on dead wood was collected from Benin Southern Nigeria (Benin 6 º20’0’’N, 5º3720’’E). Both wild and mutant strain were taken to the laboratory and cultured to obtain pure culture. Potato dextrose agar was used for the isolation of the pure culture. Preparation of the medium was according to the protocol of the manufacturers of the medium.

Mutation Induction

The actively growing culture (7 day old) of A. polytricha (EW1) was exposed to UV- light of 210nm (UV sterilizer, Millipore xx63 70000, USA) for 90 min to induce mutation. The mutant (EW1M1) was sub cultured on a PDA medium, supplemented with 5% yeast extract agar (YEA) and incubated at 25º C for 7days.

Biosynthesis of silver nanoparticles

Two grams of fresh sample of both the wild strain (EW1) and the mutant (EW1M1) of the A. polytricha were washed thoroughly with double distilled water, boiled for 10 minutes and filtered through whatman No1 filter paper using the method of [14] with slight modification. The extract was stored at 4 oC for further use.

The filtrate was used as reducing and stabilizing agent for 1mM of AgNO3 (99.9% Sigma- Aldrich). 1µl of the mushroom extract at a time was added to 100 ml of 103AgNO3 aqueous solution (prepared in deionized water), boiled and incubated at 37ºC until the colour changed to brown. Mushroom extract without the aqueous solution of AgNO3 was boiled in deionized water and served as the control experiment.

UV visible spectroscopy analysis [14]

The process of reaction between metal ions (silver ions) and biosynthesis of silver nanoparticle by the mushroom species (Auricularia polytricha) was monitored by UV visible spectroscopy of the aqueous solution. UV visible spectrophotometer (Spectroquant ® Pharo 300 M, UV) with a resolution power of 2.0 nm at between 200 to 600 nm, possessing a scanning speed of 300 nm / minutes was used for the analysis.

Fourier transmission infrared spectroscopy measurements (FTIR) [14]

Fourier transmission infrared spectroscopy measurements (FTIR) were carried out to determine the different functional groups present in the resulting silver nanoparticles. This was done following the method of [14] The residual solution after the biosynthesis reaction was centrifuged at 10,000 rpm for 15 minutes three times to purify the suspension by removing proteins /enzymes. The sample was completely dried at 60ºC. Finally, the dried nanoparticles were analoged by FTIR The Fourier transform infrared spectroscopy (FT-IR) spectrsum of the sample was recorded on Perkin Elmer spectrum 100 FT-IR spectrometer using KBr. (Thermo Nicolet nexus 670 spectrometer of resolution 4cm -1). Two grams of fresh sample of both the wild strain (EW1) and the mutant (EW1M1) of the A. polytricha were washed thoroughly with double distilled water, boiled for 10 minutes and filtered through whatman No1 filter paper using the method of [14] with slight modification. The extract was stored at 4 oC for further use.

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Dynamic Light Scattering (DLS)

The DLS was used to determine the average size distribution of the synthesized AgNPs. The reaction mixture was filtered several times and the resulting pure suspension put in an Ependoff tube. The particle distribution in the liquid was studied in a computer-controlled particle analyser (ZETA sizer Nanoseries Malveron instrument Nano Zs).

Transmission Electron Microscopy (TEM)

The transmission electron microscopy analysis was used to measure the size, morphology and chemical composition of the silver nanoparticles produced. The sample to be analysed were prepared by several layers of coating and allowed to dry. TEM images were obtained at an accelerating voltage of 300 kV.

Test Organisms

Two human pathogenic bacteria (Staphylococcus aureus and Escherichia coli) and two species of fruit rotting fungi (Aspergillus flavus and Penicillum notatum) were chosen as test organisms to determine the antimicrobial activity of the resulting AgNPs. Pure cultures of these organisms were obtained from the Microbial Biotechnology laboratory of the Department of Biotechnology (DBT-Centre) of Assam Agricultural University (AAU), Jorhat, India. The bacterial strains were maintained on nutrient agar (NA) medium at 35ºC until they were used, while the fungi species were maintained on Potato Dextrose Agar (PDA) (M-Lab) at 25ºC.

Antibacterial Activity [9]

The antibacterial activity of the synthesized silver nanoparticle, mushroom extract and the control were tested against the selected bacterial strains mentioned above using the method of [9]. Twenty millilitres (20 ml) of sterilized nutrient agar medium was poured into sterile petri plates and allowed to solidify. The test bacterial cultures were evenly spread over the medium by using a sterile cotton swab. A well of 0.5cm was made in the medium using a sterile cork borer. 200 µl of synthesized silver nanoparticle, mushroom extract and the control were each filled into a well on the plate and the culture was incubated for 24 hrs. At the end of the incubation period the plates were checked for clear zone of inhibition around each well.

Antifungal Activity

The antifungal activity of the synthesized silver nanoparticle and mushroom extract were tested against selected fungal strains mentioned above. The agar well method as described by [9] was employed in the assay.

Results

Biosynthesis of silver nanoparticles

There was a gradual visible colour change from light brown colour to reddish brown, when the mushroom extract was subjected to different concentrations of aqueous solution of 1mM of silver nitrate. This indicated the formation of silver nanoparticles in the mushroom extract.

UV vis spectroscopy analysis

Metal nanoparticles such as AgNPs had free electrons, which gave rise to a surface plasmon resonance (SPR) absorption band. The absorption spectra of AgNPs exhibited a strong broad peak at 420 nm, and observation of this band was attributed to surface plasmon resonance of

the particles which confirmed the presence of silver (Figure 1).

Fourier transmission infrared spectroscopy measurements (FTIR)

FT-IR study revealed the absorption bands at 3380, 2921, 2839, 1658, 1083 and 610 cm¯1 showing O-H(stretching), C-H(stretching), Aldehyde, C=O(stretching) Amide, N-H(stretching) Amide, C-Cl Alkylhalide respectively indicating the functional groups reducing the silver nitrate to silver ion.

Dynamic Light Scattering (DLS)

The particle size distribution analysis revealed that particle size of the silver nanoparticles produced in this study was between 5-50 nm (Figure 3).

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) image analysis conducted showed that the morphology of AgNP2 produced was spherical with a size of 5nm (Figure 4). The nanoparticles produced were monodispersed.

Antibacterial/Antifungal Activity

Both the ordinary mushroom extracts and the synthesized nanoparticles exhibited antimicrobial properties. The synthesized silver nanoparticles of the mutant strain (EWIMI) elicited a higher antimicrobial activity against the test organisms strain (EWI) (Table 1).

Discussion

The gradual colour change from white/creamy to brown observed when the mushroom extract was treated with silver nitrate indicated the formation of silver nanoparticles. The observed colour change was as a result of excitation of surface plasmon resonance in the synthesized silver nanoparticles. [18] gave similar reports when they studied the biosynthesis of silver nanoparticles by Agaricus bisporus. A similar finding was also reported by [19] using Agaricus bisporus, Calocybe indica, Pleurotus florida and P. platypus extract.

The synthesized AgNPs of A. polytricha were characterized by UV-Vis Spectroscopy. There was a strong broad band absorption spectrum at 420 nm. This result is in agreement with the reports of [19] who studied the synthesis of silver nanoparticles from edible mushroom (Agaricus bisporus). Similar observation was also reported by [10] in their study of extracellular nanoparticles synthesis using Pseudomonas aeruginosa. This shows that mushrooms could be veritable tool in the synthesis of silver nanoparticles which have been shown to possess strong antimicrobial properties against some pathogenic organisms. The DLS shows the average size of synthesized AgNPs distribution analysis.

TEM micrographs of the nanoparticles obtained in this study show that the synthesized AgNPs are spherical in shape with average size of 5-50 nm. The AgNPs were monodispersed and well distributed within the solution. [16] in their study of AgNPs synthesis using Pleurotus florida reported the synthesis of AgNPs of average size of 20 nm that was polydispersed. However, [15] recorded the AgNPs of size range of 5 to 50 nm using Pleurotus sajo-caju

In this study, our FTIR investigation of the resulting AgNPs revealed absorption bands at 3380, 2921, 2839, 1658, 1083 and 610 cm1¯ representing Hydroxyl group O-H stretching, aldehyde

C-H stretching , C=Ostretching, amide I group and amide

II groups respectively. Similar findings have been reported by [7]. The peak at 1534cm-1 is attributed to amide II vibrations of proteins [13]. Amide II bands along with amide I bands were major regions of the protein infrared spectrum. The major absorbance peak at 1083 cm-1is attributed as structures in

chitin, a major structural polysaccharide in mushrooms; and this absorbance at 1083 cm-1may also arise from primary alcohol structures due to alcohol functional group detected [21]. The peak band at 610 cm-1 corresponds to the presence of alkyl halides.

The antimicrobial activity of the silver nanoparticle of A. polytricha was investigated against some pathogenic organisms using well diffusion technique. The synthesized silver nanoparticles recorded higher antimicrobial activity than the mushroom extract. This shows that the silver nanoparticle could be used as antimicrobial agents against the human pathogens that have been tested. Similar findings have been reported by [19]

Conclusion

The results of this study show the improved performance of mutant strain of Auricularia polytricha EM1W1 over its wild type EW1. Absorption spectra of silver nanoparticle (AgNPs) of mutant exhibited a strong broad peak at 420 nm while wild type absorption peak was obtained at less than 420 nm. The formation of zone of inhibition on all the microorganisms that were used for the study shows the usefulness of AgNPs in medicine while the control showed no zone of inhibition. The mushroom extract of mutant strain exhibited higher anti microbial activity than the wild type.

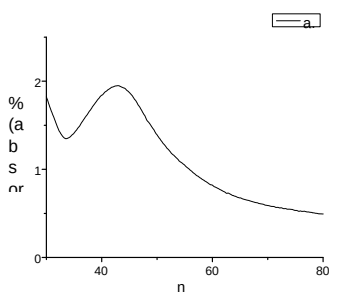

Fig 1: The *Auricularia* (EW1

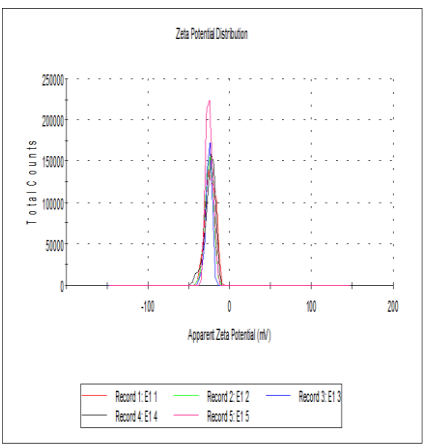

Fig 3: The DLS of *A. polytricha* showing peak

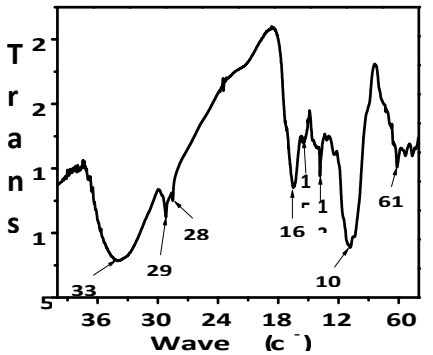

Fig 2: The FTIR from silver synthesi*Auricularia*

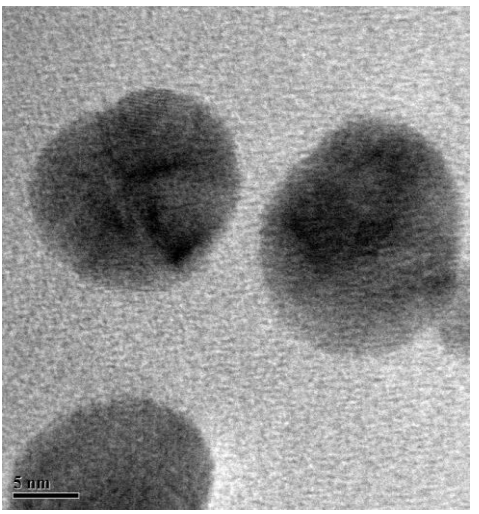

Fig 4: The TEM analysis of silver nanoparticles synthesized by Auricularia polytricha

Table 1: The mean zone of inhibition (mm) of Auricularia polytricha isolates.

Tested organisms	Zone of inhibitions (mm)				
	Synthesized silver nanoparticles		Mushroom extract		Control
	EW1	EW1M1	EW1	EW1M1	
P. acidiovorans	9.0	11.0	8.0	8.6	-
Escherichia coli	3.5	5.0	2.5	2.0	-
Staphylococcus aureus	2.0	3.5	0.5	0.0	-
Bacillus cereus	9.0	12.0	7.0	7.9	-
Aspergillus flavus	4.0	6.0	2.0	2.0	-
Penicillum notatum	1.0	2.0	0.5	0.9	-

Key – means no zone of inhibitions

Acknowledgements

Authors are grateful to the Faculty and staff members of Agricultural Biotechnology Department, Assam Agricultural University, Jorhat, India for providing the facilities used in this research. The World Academy of Sciences (TWAS), Italy is greatly acknowledged for funding this research and the Management of Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos Nigeria is appreciated for opportunity to utilise the award.

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