



## RESEARCH ARTICLE

# Biogenic Synthesis of Silver Nanoparticle from Mushroom Exopolysaccharides and its Potentials in Water Purification

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### Abstract:

#### Objective:

This study reports a novel eco-friendly biosynthesis of Silver Nanoparticles (AgNPs) from Exopolysaccharides (EPS) of *Lentinus edodes* after an attempt to optimise the production of EPS through mutagenesis. It further describes some potential application of silver nanoparticles in water treatment.

#### Methods:

A wild strain of *L. edodes* was subjected to UV irradiation, a physical mutagen, at 254 nm. The wild and resultant irradiated strains were then assessed for the production of EPS and subsequent application of the crude EPSs for biosynthesis of AgNPs. The particles were characterised by colour pattern and UV-visible spectroscopy. Based on superior EPS production and nanoparticle attributes, nanoparticles obtained from UV irradiated process were further subjected to Scanning Electron Microscopy (SEM). EPS produced was quantified by the phenol-sulphuric acid method and studied by GC-MS.

#### Results:

Results obtained for EPS productivity indicated the presence of monomer sugars such as arabinose (50.65%), mannose (19.20%), mannitol (15.58%), fructose (7.96%), trehalose (6.49%), and glucuronic acid, xylose, galactose and glucose with low percentages of  $\leq 0.11$ . EPS productivity of wild and mutant strains was obtained as 1.044 and 2.783 mg/ml, respectively, after 7 days of fermentation. The result of EPS production for UV irradiated strain corresponds to a yield improvement of 2.7 fold of the wild-type. UV Spectroscopy and SEM analysis studies on EPS nanoparticle product of the improved (UV irradiated) strain indicated the formation of AgNPs at the absorption band of 421 nm with a size range of 50-100 nm.

#### Conclusion:

This study, which aimed at eco-friendly synthesis of myco-nanoparticle has established the novel ability of *L. edodes*' polysaccharide in silver nanoparticles biosynthesis. It expounded potential frontiers of silver nanoparticles application in the water industry. To the best of the authors' knowledge, this result represents the first report on the biosynthesis of AgNPs using *L. edode*'s EPS.

**Keywords:** AgNPs, EPS, Mutants, *L. edodes*, UV irradiation, Myco-nanoparticle.

## 1. INTRODUCTION

Nanotechnology is the study, construction and utilisation of materials in the physical range of 1-100 nm [1]. Nanoparticles are regarded as the basic building blocks of nanotechnology and the development of a biological process for the synthesis of nanoparticles is developing into an important branch of nanotechnology [2, 3]. In the recent decades, nanoparticles have met a great expansion and serious investigations due to their potentials in a wide spectrum of industrial applications [4, 5]. Biologically synthesized nanoparticles have been implicated in the production of

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antibacterial and antiviral materials, catalysts in biological labeling, biosensors, chemical reactions, detection of genetic disorders, drug delivery, electrical batteries, gene therapy and DNA sequencing, optical receptors [6].

In order to meet the challenge of water purification, several techniques like adsorption, biosorption, electrochemical treatments, evaporation, flotation, ion exchange, membrane filtration, oxidation, precipitation and reverse osmosis processes are extensively used [7 - 9]. Such previous applications have been restricted due to various shortcomings [10, 11]. Research is evolving to use high-tech nanotechnology in water treatment for safe drinking. Nanoparticles are expected to be of vital importance in water treatment technology of the future [12] for which research is still at infancy. Developments in nanoscale science and engineering suggest that many of the existing water quality challenges could be resolved or greatly diminished by using bioactive nanoparticles, nanoadsorbents, nanocatalysts, nanostructured catalytic membranes, nanotubes, magnetic nanoparticles and submicron nanopowder. Nanotechnology and its science have been used for detection of algae (*e.g.* cyanobacterial toxins), antibiotics and biological agents, (like bacteria, parasites and viruses), biological and chemical substances including metals (*e.g.* cadmium, copper, lead, mercury, nickel, zinc), cyanide organics, nutrients (*e.g.* phosphate, ammonia, nitrate, nitrite), as well as pesticides [13]. The large surface area of nanoparticles, catalytic potential and high reactivity, makes them better adsorbing materials than conventional treatment technologies [10]. Some advantages and disadvantages of previous water treatment methods are provided in Ali *et al.* [11]

Nanoparticles have been conventionally produced by chemical and physical methods [14] which involve techniques like heating [15] and irradiation [16, 17]. However, such practices are costly, toxic and hazardous [18] though such methods have been used to achieve various desired results including size modification [19]. Hence the need for alternative, eco-friendly approaches, based on biological methods. There are documented reports that the techniques of nanoparticles synthesis through biological means make such products more biocompatible and environmentally friendly. Applying biological methods for the production of nanomaterials have received extensive attention because the techniques are not expensive and are eco-friendly; also, biosynthesis of nanoparticles can be carried out in one step [20]. Green synthesis methods utilise miscellaneous biological natural substances such as marine algae, microfluidics, microorganisms like bacteria, fungi, yeasts and diatoms, plant tissues and fruits, plant extracts and whole plants for the reduction and stabilisation of nanoparticles. Synthesis of nanomaterials using extracts of plants and related materials has numerous advantages over other environmentally green synthesis approaches, because plants are easily available, largely distributed, less expensive, readily scalable and safe to handle [21]. Among various nanoparticles, silver nanoparticles, due to their properties such as potent antimicrobial activity, catalysis and electrochemical conductivity can be used in different applications like agriculture, biomedicine, food chemistry and photo chemicals [22, 23].

Some comparative studies of chemically and biologically synthesised nanoparticles are presented in Table 1 (antibacterial characteristics) and Table 2 (particle size and distribution characteristics). The presented results revealed that biological methods for nanoparticle synthesis, in addition to being eco-friendly, are comparatively similar and sometimes better in performance to chemically synthesised nanomaterials.

Fungi like mushroom are choice biomaterial for nanoparticle synthesis as they are easy to handle, culture and possesses high wall-binding capacity as well as intracellular metal uptake capabilities [26]. These characteristics may therefore enhance processes and performances of myconanoparticles. Various fungi and fungal extracts have been used for biosynthesis of AgNPs [27, 28] which are currently being explored for safe water purification. The interface of mycology and nanotechnology is termed myconanotechnology [29 - 31].

**Table 1. Antibacterial activity of silver nanoparticles synthesised by chemical and biological methods.**

Organisms	Zone of Inhibition (mm)		
	Biological AgNPs	Chemical AgNPs	Standard
<i>Staphylococcus aureus</i>	25	20	21
<i>Bacillus subtilis</i>	24	19	21
<i>Escherichia coli</i>	26	20	25
<i>Klebsiella pneumoniae</i>	22	18	23

Source: Gudikandula and Maringanti (2016) [24].

This paper reports the mutagenesis of *L. edodes* for EPS yield enhancement and the application of the produced EPS in biological synthesis of silver nanoparticles (AgNPs) as eco-friendly alternative in bionanotechnology. It highlights some application area of nanomaterials in emerging water quality treatment technologies. The EPS produced was

characterised by GC-MS while AgNPs was typified by visual colour pattern, UV-Visible spectroscopy, and Scanning electron microscopy.

**Table 2. Properties of nanoparticles produced by thermal treatment at different temperature compared with bisynthesised nanoparticles.**

T (K)	Size (nm)	Particle Size Distribution
273	35.1	43-143
283	23.2	40-114
293	17.6	38-91
303	16.5	30-57
Bio-nanoparticle	17.6	35-120

Source: Kahani and Yagini (2014) [25].

## 2. MATERIAL AND METHODS

### 2.1. Collection of *L. Edodes* Strain

A pre-typified wild *L. edodes* was obtained from Mushroom Research Centre, Himachal Pradesh, India. This was reproduced by tissue culturing and maintained by sub-culturing monthly and stored at 4°C on Potato Dextrose Agar (PDA) slants to maintain viability.

### 2.2. Induction of Physical Mutation

The mutagenesis process was carried out by irradiating 14-day actively growing culture of the wild fungus on PDA plate (90 mm) with ultraviolet irradiation (254 nm, 15 cm) between the ranges of 5 to 90 mins. Mutants were obtained at 5, 10, 15, 20, 25, 30, 45, 60, 75 and 90 mins and were instantly sub-cultured and incubated in the dark at 26 ± 2°C for 2 weeks. Based on improved (EPS) productivity (assessed using the method of Dubois *et al.* [32]), a resultant viable mutant obtained at 10 mins of UV irradiation (UV10) was carefully chosen. This culture was incubated at 25 ± 2°C for 70 hours and subsequently re-introduced onto the newly prepared PDA slant, incubated for 2 weeks to obtain fully ramified UV10 strain. The wild and UV10 strains were re-evaluated for quality enhancement and used for the production of EPS using combined methods of Adebayo *et al.* [33] and Majolagbe *et al.* [34].

### 2.3. EPS Production, Extraction and Quantification

Exactly 14 day old UV10 strain was used to inoculate 50 ml of production media in 250 ml conical flask consisting in g/L of glucose (10.0), KH<sub>2</sub>PO<sub>4</sub> (0.5), MgSO<sub>4</sub> (0.5), peptone (2.0) and yeast extract (1.0). Other fermentation conditions include agitation (100 rpm), temperature (27 ± 2°C), and incubation time of 7 days. Crude EPS was obtained as cell free extract after filtration and centrifugation. Extraction of EPS was carried out by mixing cell free supernatant from the respective production medium with 2 volumes of cold absolute ethanol (v/v) and held at 4°C overnight for EPS precipitation. EPS precipitate was collected as crude fraction after centrifugation at 4000 rpm for 15 min (AG 5702, Eppendorf, Germany) using the protocol of Majolagbe *et al.* [34] and re-suspended in 20 ml of sterilised distilled water. The quantity of EPS produced per millilitre of distilled water was evaluated by UV spectrophotometry. Absorbance at 490 nm of digested EPS was obtained per extract using phenol-sulphuric acid method of Dubois *et al.* [32] and calibrating to total sugar contents using glucose as standard. EPS sugar monomer compositions of the obtained polysaccharides were subsequently assessed using GC-MS (GC2010, Shimadzu, Japan) [35].

### 2.4. Calibration Using Reducing Sugar Standard

Exactly 0.1 g of anhydrous glucose, as obtained from Sigma Aldrich (South Africa), was used for preparation of working standard solution and dispensed into Erlenmeyer flask, liquefied in 19 ml of distilled water, and then made up to 100 ml, making dilution factor of 10<sup>-2</sup> (1 mg/ml). Then, 2, 3, and 5 ml of the stock solution was diluted to 100 ml to give working standards of 0.02, 0.03, and 0.05 mg per ml respectively. Subsequently, 1 ml was taken from the working solutions into separate 50 ml tube and 1 ml of 5% phenol was added, followed by the addition of 5 ml of concentrated H<sub>2</sub>SO<sub>4</sub> added rapidly and then allowed to cool. The absorbances of admixtures were then read at 490 nm on a UV-visible spectrophotometer (Genesys 10UV scanner, Thermo electron corporation, UK), and used to plot a calibration curve for the determination of sugar content.

## 2.5. Determination of EPS Contents in Fermentation Broth

Specifically, about 1 ml of the re-dissolved EPS in distilled water (stock) was measured in a test tube and 1 ml of 5% phenol solution was thereafter added, followed by the rapid addition of 5 ml of concentrated sulphuric acid and then allowed to cool. The absorbance for each EPS product of all the UV mutants were therefore obtained at 490 nm and quantity determined using the calibration curve obtained.

## 2.6. EPS-mediated Synthesis and Characterization of Silver Nanoparticles (AgNPs)

Silver NPs were synthesised by reacting crude EPS of UV10 with 1 milli molar solution of silver nitrate. Exactly 1 ml of EPS solution of the Wild and UV10 was dispensed into 5 ml 1mM AgNO<sub>3</sub> in a 15 ml capacity test tube. A control was set up in a third test tube which contained 6 ml of 1 mM AgNO<sub>3</sub>. The 3 tubes were strongly shaken for about 10 minutes and examined for colour development at room temperature (28 ± 2°C) during intermittent shaking. The formation of colour as a result of AgNPs development was monitored visually and the absorbance spectrum of the reaction solution was measured and recorded on a UV-visible spectrophotometer (Genesys 10 UV, Thermoelectron Corporation, UK). Morphology of the biosynthesised silver nanoparticles was elucidated by Scanning Electron Microscopy (SEM). SEM images were collected using an ASPEX 3020 at an accelerating voltage of 15 kV in bright field mode.

## 2.7. Comparing EPS and AgNPs UV-Visible Studies of Wild and UV10 Mutants

The exopolysaccharide productivity study was according to the method of Dubois *et al.* [32] and Majolagbe *et al.* [34]. UV Visible and colour development pattern studies for AgNPs production were carried out to observe the surface plasmon resonance and typical silver nanoparticle colouration respectively. The results obtained were assessed for comparative efficiencies of the resultants EPSs and nanoparticle produced.

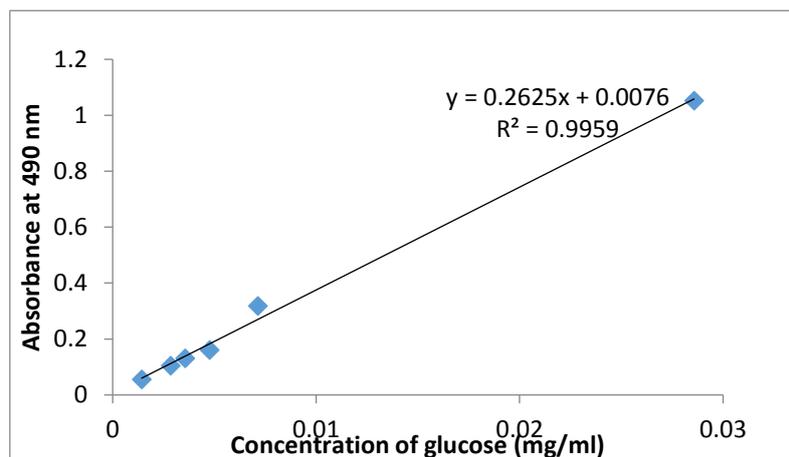
## 3. RESULTS AND DISCUSSION

### 3.1. EPS Yield and Sugar Component Elucidation

UV10 culture resulting from 10 minutes of exposure to ultraviolet irradiation gave EPS productivity of 2.783 mg/ml at 7 days of fermentation using the sugar calibration curve in Fig. (1). This was done by inserting the absorbance reading obtained from UV-vis spectroscopy of UV10 into the regression equation. The monosaccharide sugar composition of the produced EPS was identified as shown in Table 3. The results indicated the presence of monomer sugars that were characteristic of exopolysaccharides including arabinose (50.65%), mannose (19.20%), mannitol (15.58%), fructose (7.96%), trehalose (6.49%), and glucuronic acid, xylose, galactose and glucose with low percentages of ≤ 0.11. The results therefore indicate that the EPS produced is mainly made of arabinose, mannose and mannitol. It confirms the potentials of *L. edodes* for applications in production of polysaccharides with industrial usefulness. According to Sutherland [36], monosaccharide components most classically found in EPS are sugars such as amino sugars (D-Galactosamine and D-Glucosamine), hexoses (D-Mannose, D-Glucose, D-Galactose, D-Allose, L-Rhamnose, L-Fucose), pentoses (as D-arabinose, D-Ribose, and D-Xylose), or uronic acids (D-Galacturonic acids and D-Glucuronic acids). Thus, these results are mostly in accordance with those previously reported [36 - 38].

**Table 3.** EPS sugar monomer compositions.

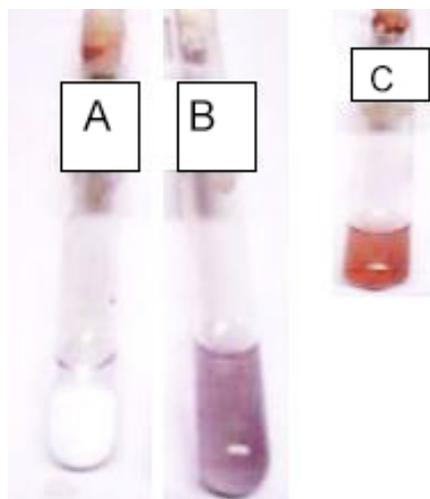
Sugars	Concentration mg/100 g	Relative molar percentage (%)
Arabinose	1535.7500	50.6472
Mannose	582.2155	19.2008
Mannitol	472.4787	15.5818
Fructose	241.4632	7.9632
Trehalose	196.9211	6.4942
Glucuronic acid	3.3352	0.1100
Xylose	0.0367	0.0012
Galactose	0.0306	0.00100
Glucose	0.0200	0.00066



**Fig. (1).** Calibration curve for polysaccharide content determination.

### 3.2. Colour Change

Aqueous silver nitrate ions were reduced during exposure to the EPS extract of UV10. The colour of the reaction mixture changed from colourless to purple with observed colour pattern getting stable after 24 hours as shown in Fig. (2) (Wild in-set). Purple and yellowish-brown colouration have been reported in relation with nanoparticle synthesis and due to excitation of surface plasmon vibration in metal nanoparticle [39]. Characteristic AgNPs colouration earlier reported include yellowish brown [40], yellow and reddish brown [41], light-gray [42], and dark brown [43]. Observed variation in colour pattern has been attributed to the differences in composition of biomolecules used in nanoparticle synthesis and the excitation of surface Plasmon vibrations in metal nanoparticles [44]. Time for development of AgNPs reported by some authors include 10 hrs [40], 24 hrs [43], and 48 hrs [41]. In this study EPS of *L. edodes* rapidly reacted with  $\text{AgNO}_3$  solution to form AgNPs in 24 hrs.



**Fig. (2).** AgNPs Colour patterns of Control (A) and UV10 mutant (B) after 24 hours. Inset is the colour pattern of AgNPs of Wild (C).

### 3.3. UV Visible and SEM Studies of AgNPs Produced by UV10

UV-visible absorption spectroscopy has proved to be a versatile technique in the studies of AgNPs [45] and provides useful information about morphology, size and stabilisation of AgNPs [46]. Fig. (3) illustrates the UV-visible spectra of biosynthesised AgNPs of UV10 strain after 24 hours (Wild inset). The result shows a broad Surface Plasmon Resonance (SPR) with peak attained at 421 nm. The UV spectrophotometry reported in this study falls within the range of a usual and well reported pattern of SPR peaks of silver nanoparticles production [47 - 51]. These characteristic AgNPs absorption peaks justified the formation of AgNPs. Several authors have also reported peaks on spectra of

AgNPs in the range of 391-650 nm [50 - 52]. According to some established protocol by Brennan *et al.* [52], silver particles which displays single absorption band and occur between the range of 410 and 450 nm are spherical in nature. The silver particles produced by UV10 in the wavelength of 421 nm can therefore be presumed to be spherical in nature. Peaks in this range are further supported by other reports to be characteristics of spherical or somewhat spherical shaped nanoparticles [39, 42, 53]. These further substantiates the thought that extracts of UV10 produced nanoparticles that are somewhat of spherical morphology under the conditions investigated. The outcome of nanoparticles produced in this work is similar to those previously reported by different authors [21, 42, 53 - 55] with respect to the production of silver nanoparticles using whole cells and extracts of mushrooms and other plants.

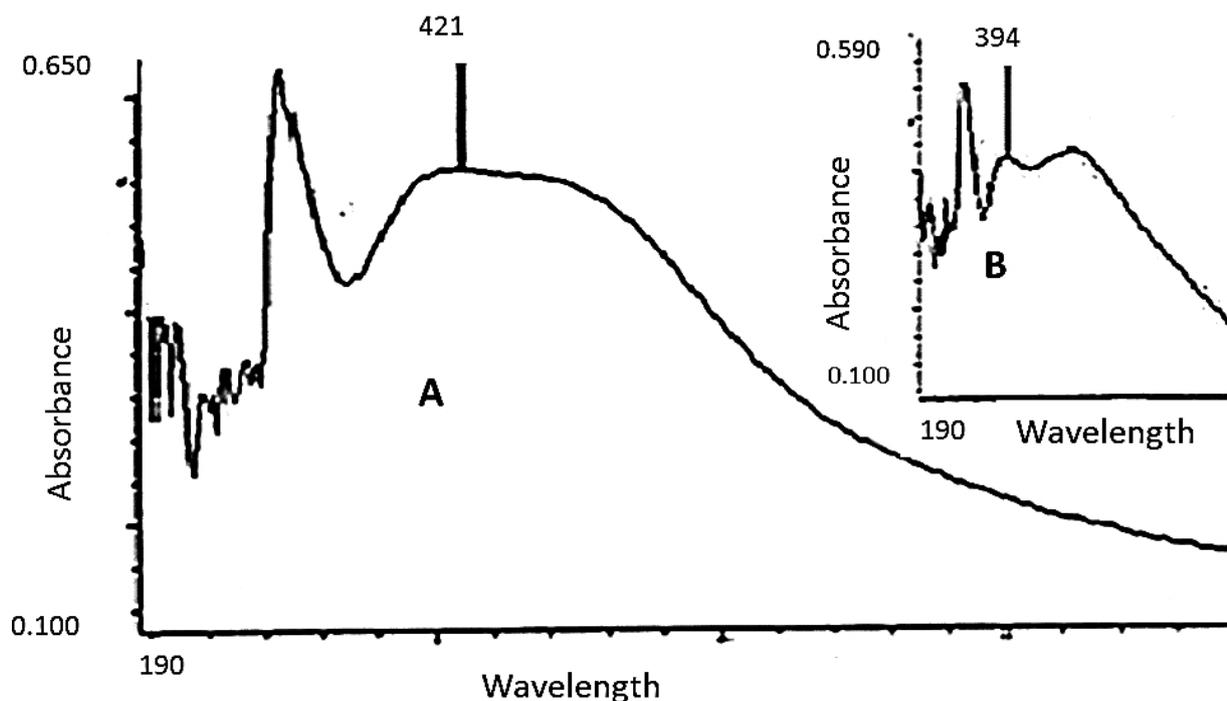


Fig. (3). UV-Visible spectra of AgNPs of UV10 (A) after 24 hours. Inset, the spectra of UV-Vis spectra of AgNPs of Wild (B).

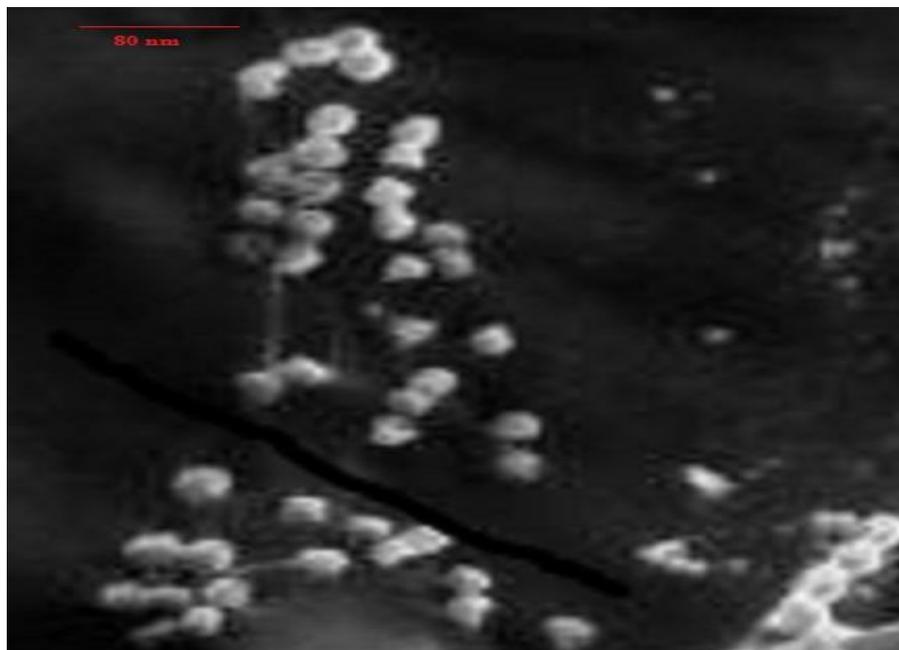
### 3.4. SEM Characterisation of Synthesised Nanoparticles

The scanning electron microscopy implemented to confirm the morphometric physiognomies of shape and size of the bio-nanoparticles showed that nanoparticles produced contained aggregates of particles with spherical morphology (Fig. 4). The size ranges between 50-100 nm. Spherical shaped AgNPs of varying sizes have been earlier described [51, 56, 57]. Nanoparticles have been reported to possess high reduction potentials, good solution chemistry as well as extremely small particle size with large surface area [58]. The large surface area of nanoparticles as a result of nano-sizes provides a greater number of active sites for pollutants binding. In addition, the particles demonstrate unique characteristics like catalytic potential and high reactivity, which make them better adsorbing materials [10]. Moreover, high density of low coordinated atoms at the surface edges of nanomaterials further makes them very reactive [59]. These distinctive properties can be employed in the adsorption and degradation of water and air pollutants. The successful synthesis of biologically mediated nanomaterial using EPS of *L. edodes* in this study may therefore herald a simple novel technique of biosynthesis and modulation of nanoparticles of varied sizes and properties. Nanoparticle of varied sizes such as synthesised can be applied in diverse industries, especially, in the field of hydrology as highlighted in this report.

### 3.5. Comparing EPS Productivity, AgNPs Colour and Spectra pattern of Wild and UV10 Strains

Comparing the EPS productivity of the Wild and UV10 mutant strains of *L. edodes*, UV10 obtained at 10 minutes of exposure to Ultraviolet irradiation gave the highest exopolysaccharide productivity of 2.783 mg/ml while the Wild strain has a productivity of 1.044 mg/ml after 7 days of fermentation (Table 4). The 10 minutes UV improved strain has an enhanced capacity of 2.7 fold over the Wild strain, which confirms the effectiveness of UV irradiation for EPS

productivity enhancement in the mushroom strain. The synthesised nanoparticle of UV10 has a purple colouration and detected around 421 nm while that synthesised by wild strain possesses dark brown colouration with absorbance of 394 nm which are significant nanoparticles formation characteristics [19].



**Fig. (4).** Silver Nanoparticle synthesised from UV10.

**Table 4.** Comparative activities of UV10 and Wild.

Strains	Sugar (mg/mL sample)	Fold of Exopolysaccharide	AgNPs Colouration	AgNPs Spectra Readings (nm)
Wild	1.044	-	Dark brown	394
UV10	2.783	2.7	Purple	421

### 3.6. Potential Water Applications

#### 3.6.1. Water Disinfectants

The antimicrobial activities of silver have been known since ancient times and used efficiently against a wide range of microorganisms [60]. Silver nanoparticles (AgNPs) are highly lethal against microorganisms and thus have strong antimicrobial effects against wide spectra of microbes, including bacteria [61], fungi [62] and viruses [63]. Silver nanoparticles are able to adhere to microbial cell wall and consequently penetrate it, causing structural changes of the cell membrane and thus making it more permeable [64]. Furthermore, when AgNPs are in contact with microbes, free radicals can be produced which can destroy the cell membrane leading to cell death [65]. In addition, as DNA contains abundant sulfur and phosphorus elements, AgNPs may react with those elements and thus destroy them; and this further explains death of cells caused by AgNPs [66]. The dissolution of AgNPs will release antimicrobial  $\text{Ag}^+$  ions, which may react with the thiol groups of several important enzymes, deactivate them, and interrupt regular functions in the cell [67]. As a good antimicrobial agent, silver nanoparticles, under suitable conditions such as effective magnetic separation, would therefore be a good antimicrobial disinfectant in water treatment.

#### 3.6.2. Water Filtration Materials

AgNPs blended with filter materials is considered promising for water purification due to their high antimicrobial activity and low cost. This procedure is expected to overcome the challenge of aggregation in aqueous media that progressively decreases efficacy of nanoparticles during long-term use [68, 69]. AgNPs deposition on cellulose fibers of an absorbent blotting paper sheet has been reported. The AgNPs sheets showed antibacterial action against suspensions of *Enterococcus faecalis* and *E. coli* by inactivating cells of the bacteria strains during filtration through the sheet [70]. The silver loss from the AgNPs embedded sheets was lesser than the standards for silver in potable water as recommended by the World Health Organization (WHO) and Environmental Protection Agency (EPA) [70]. In

addition, silver nanoparticles incorporated into Polyethersulfone (PES) microfiltration membranes was reported to remarkably inactivate the activity of microbes near the membranes. The PES-AgNPs membranes showed strong antimicrobial characteristic and indicate great potential in the application of silver nanomaterials for the development of potent water filters for safe water processes [71].

### 3.6.3. Anti-Fouling Agent

Ag nanoparticles on clay materials have drawn significant attention as a result of their anti-fouling properties for domestic (point-of-use) water treatment [72]. Silver nanoparticle's application to ceramic filters fabricated with sawdust and clay have exhibited improved water treatment efficiency in this aspect with filters of higher porosity attaining greater application potentials than those of lesser porosity [73]. Antifouling properties of silver nanoparticles regenerated by TiO<sub>2</sub> on forward osmosis membrane have been reported [74] while nano-silica fabricated silver nanoparticles have also been reported as an effective antifouling agent in addition to aiding dye removal and disinfection in polluted water [75].

## CONCLUSION

In this study, results obtained confirm that an alternative technique for synthesis of silver nanoparticles using biological approach is feasible. To the best of our knowledge, this is the first report of using mutagenesis in biosynthesis for modulation of product of nanoparticles. This procedure may be a useful tool in the production of different types of nanoparticles and may constitute a useful technique in the amplification of eco-friendly and biologically driven processes for safe drinking water treatment and production.

## RECOMMENDATION

The potentials of silver nanoparticles in water quality improvement have been highlighted in this work. Biological production and simple UV optimization technique for AgNPs of mushroom origin is hereby established. This cheap method of myco-nanoparticle production and yield improvement can further be extended to the range of underutilised mushrooms, especially, for water research and application purpose.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies in this research.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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