International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 12 (2013) pp. 605-614**
http://www.ijcmas.com

Original Research Article

Studies on biosynthesis of silver nanoparticles using mushroom and its antibacterial activities

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A B S T R A C T

Silver nanoparticles play a significant role in the field of biology and medicine. Silver nanoparticles were synthesized rapidly within 72 hour of incubation period using *Agaricus bisporus*, *Calocybe indica*, *Pleurotus florida*, and *P. platypus* extract. While the mushroom extract incubated with deionized water retained its original colour. The silver nitrate treated mushroom extract turned to brown colour after 72 hours due to deposition of silver nanoparticles. UV – visible studies indicated the surface Plasmon resonance at 300nm which depicts the formation of silver nanoparticles. The FTIR studies showed the presence of functional groups involved in the reduction of silver nitrate to silver ions. Scanning electron microscopy has provided further insight into the morphology and size detail of the synthesized nanoparticle. SEM micrograph revealed the formation of polydispersed nanoparticles. The silver nanoparticles of *Pleurotus platypus* wasfound to have wider antibacterial activity in all bacterial cultures. The maximum zone of inhibition was observed in *Enterobacteraero genes*(18mm). The mushroom extract showed moderate activity against *Enterobacteraerogenes*(14mm). In control there was no zone formation in all bacterial strains. The high bactericidal activity is certainly due to the silver cations released from Ag nanopaticles that act as reservoirs for the Ag^{+} bactericidal agent.

K e y w o r d s

Mushroom;
Antibacterial activity;
UV;
FTIR;
SEM.

Introduction

Mushroom is the fleshy, spore-bearing fruiting body of a fungus, typically produced above ground on soil or on its food source. The most popular of these, *Agaricus bisporus*, is considered safe for most people to eat because it is grown in controlled, sterilized environments.

Several varieties of *A. bisporus*, *Pleurotus platypus*, *P. florida* and *Calocybeindica* are grown commercially, including white, crimini, and portobello. Nanotechnology is mainly concerned with the synthesis of nanoparticles of variable size, shape, chemical composition and controlled

disparity and their potential use for human benefits.

In the past decade there has been a tremendous amount of research interest in nanomaterials with respect to its production properties and applications (Narayanan and Sakthivel, 2010 and Singh *et al*., 2011). Nanoparticles are particulate dispersions or solid particles with a size in the range of 10 – 1000nm. The characteristic Physical, Chemical, Electronic, Electrical, Mechanical, Magnetic, Thermal, Dielectric, Optical, and biological properties of nanoparticles are distinct from the bulk material of the same element. Optoelectronic, physicochemical and electronic properties of nanoparticles vary with difference in their size, shape and crystalline. Therefore, the production of monodispersed nanoparticles with diverse size and shape has been a goal of numerous investigations in the field of nanotechnology.

Artificially made metal nanoparticles are typically produced on a small scale using methods such as chemical vapour deposition irradiation or chemical reduction of metal salts. However most of these processes give rise to harmful by products (Mansoori 2005). Therefore stress is laid on benign biosynthesis process which results in environmental friendly nanoparticles of biological origin. The use of microorganisms as nano – factories enables us to use simple large scale production of nanomaterials which does not give rise to toxic waste products. Microbial assisted biosynthesis of nanoparticles are therefore a rapidly progressing area of nanobiotechnology (Jaidev and Narasimha, 2010).

Mushrooms are known to have antiinflammatory, cardiovascular, antitumor, antiviral, antibacterial, hepatoprotective and hypotensive activities in biological system (Bernard Shaw *et al.,* 2005). Many varieties of naturally occurring mushrooms are found to have promising antioxidant and anticancer properties and prolong longevity (Mizuno and Mush 2000). Edible mushrooms are well known for their antioxidant, antimicrobial, anti-inflammatory, antitumor and anticancer activities (Ajith and Janardhanan, 2007).

This indicates that mushrooms could be valuable sources of antioxidant (Chen *et al.,* 2006) and antitumour compounds. For a long time in Asian countries, a variety of edible mushroom have been taken as vitamin and mineral supplements. Studies on edible mushroom have revealed, valuable activities related to biological response modification. Chemo preventative, Chemotherapeutic, immune modulatory, hypoglycemic and hypo cholestemic effects have been observed in edible mushroom diets high in mushrooms were reported (Grube *et al.,* 2001). Suppress the enzyme aromatase and the active antitumor material was suggested to be a polysaccharide (Lee *et al.,* 2003).

Materials and Methods

Sample collection

The mushroom samples such as *Agaricus bisporus*, *Calocybe indica*, *Pleurotus florida*, and *Pleurotus platypus* were collected from Oriental market, Thanjavur and brought into the laboratory for further process.

Biological synthesis of silver nanoparticles (Narasimha*et al.,* 2011)

Two grams of fresh *Agaricus bisporus, Calocybe indica, Pleurotus florida*, and *P. platypus* were washed thoroughly with

double distilled water and boiled for 10 minutes and then filtered through whatman No. 1 filter paper. The extracts were stored at 4°C for further experiments. The filterate were used as reducing and stabilizing agent for 1mm of $AgNo_3$ (99.99% Sigma – Aldrich). In a typical synthesis of silver (Ag) nanoparticles the mushroom extract were added to 50ml of 10^{-3} $AgNO_3$ aqueous solution (prepared in deionized water) and incubated in Shaker at 150rpm at 37°C. Simultaneously, a positive control was maintained with mushroom extract and deionized water used as negative control, containing only silver nitrate solution.

Selection of bacterial strains

Totally five pathogenic bacteria were selected for the present investigation. Among them five were bacterial strains such as *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes,* and *Pseudomonas putida*. The bacterial strains were originally obtained from Microbial Germplasm Culture Collection Unit (MGPCCU), Sri Gowri Biotech Research Academy, Thanjavur and used for the present investigation.

Preparation of microbial inoculums

The young bacterial cultures were prepared and used during the research period. The nutrient broth (NB) was prepared and then poured into several tubes. Then these tubes were sterilized. The pure bacterial strains were collected from the institute and then inoculated. The tubes were incubated at 37^0C for 24 – 48 hrs and the cultures were used for the experiments.

Antibacterial Activity
(Hae Kim *et al.,* 2007)

The antibacterial activities of the synthesis of silver nanoparticle, mushroom extract and control were tested against the selected bacterial strains. The 20ml of sterilized agar medium was poured into each sterile petriplates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a well of 0.5cm was made in the medium by using a sterile cork borer, 200µl of each as *Agaricus bisporus, Calocybe indica, Pleurotu sflorida,* and *platypus* (synthesis of silver nanoparticle, mushroom extract and control) were transferred into separate wells. These plates were incubated at 37^0C for 24 – 48 hours. After incubation period, the results were observed and measured the diameter of inhibition zone around the each well.

Antibiotic sensitivity test on microbes (Positive control)

The antibiotic sensitivity test using standard antibiotics (ampicillin, streptomycin and tetracycline) were analysed by the method of Bauer *et al*., (1996). The sterilized nutrient agar medium was poured into each sterile petriplates and allowed to solidify. By using a sterile cotton swabs, a fresh bacterial culture with known population count was spread over the plates by following spread plate technique. Then the selected standard antibiotic discs namely ampicillin, streptomycin and tetracycline were placed on the bacterial plates. Then, the plates were incubated for 24 hours at 37^oC. After the incubation period, the results were observed and the diameter of the inhibition zone was measured around the isolates.

Fourier transmission infrared spectroscopy measurements (FTIR)
(Narasimha*et al.,* 2011)

The residual solution after reaction was centrifuged at 10,000 rpm for 15 minutes and the resulting suspension was repeated for three times, after that the purified suspension was washed with deionized water to get pure free of proteins / enzymes which are not able to capping the silver nanoparticles. The sample was completely dried at 60°C. Finally the dried nanoparticles were analoged by FTIR (Thermo Nicolet nexus 670 spectrometer of resolution 4cm $^{-1}$).

UV – vis spectroscopy analysis
(Narasimha*et al.,* 2011)

UV – visible spectroscopy analysis was carried out on a Jasco v – 530, UV – visible absorption spectrophotometer with a resolution of 2.0nm between 200 to 600nm possessing a scanning speed of 300nm / minutes. The process of reaction between metal ions and biosynthesis of silver nanoparticle (*Pleurotus platypus)* was monitored by UV – visible spectra of silver nanoparticles in aqueous solution.

Scanning Electron Microscopy (SEM)
(Narasimha *et al.,* 2011)

For SEM the silver nanoparticle synthesized using mushroom (*Pleurotus platypus)* was allowed to dry completely and ground well to a powder specimen is normally required to be completely dry. Since the specimen is at high vaccum, fixation is usually performed by incubation in a solution of a buffered chemical fixative, such as glutaraldehyde. The dry specimen was mounted on a specimen using an adhesive epoxy resin of electrically – conductive double – sided adhesive tape and sputter coated with gold palladium alloy before examination in the microscope.

Results and Discussion

Szczepanowics *et al.,* (2010) investigated that the antimicrobial test was the evaluation of activity of silver nanoparticles against the clinically isolated organism *Staphylococcus epidermidis*, methicillin resistant and methicillin susceptible *Staphylococcus aureus*, *Pseudomonas aeuroginosa and E.coli*. These are the human pathogens responsible for many nosocomial infections and biofilm forming species. *Staphylococcus epidermis* and *E.coli* are susceptible for silver suspensions H (MICs values between 2.5 and 5μg/ml) and good activity against *Staphylococcus aureus*, both methicillin susceptible and methicillin resistant strains and *Pseudomonas aeuroginosa* (MICs 5-10μg/ml). In the present study, the synthesized silver nanoparticle shows an effective antibacterial activity against pathogens of gram positive and gram negative bacteria. In the present study cultures of *Klebsiella pneumoniae, E.coli, S.aureus, Enterobacter aerogenes* and *Pseudomonas putida* and the mushroom of *Agaricus bisporus* as well as multidrug resistant *S.aureus* (12mm) and *Enterobacter aerogenes* (12mm) were used for the antibacterial activity of nanoparticles. Zone formation was absent in all bacterial strains on the control plates. Mushroom extract (*Agaricusbisporus*) inhibited the growth of S.*aureus* (10mm) and *Enterobacter aerogeness* (10mm). There was no Zone formation in all bacterial strains tested (Table 1). Most bacterial strains are susceptible for nanosilver particle of *Calocybe indica*.

Table.1 Antibacterial activity of silver nanoparticles of *Agaricus bisporus*

Bacterial strains	Zone of inhibition (mm)		
	Synthesis of silver nanoparticles in *Agaricu sbisporus*	**Mushroom extract**	**Control**
Klebsiella pneumoniae	11	8	-
Escherichia coli	11	9	-
Staphylococcus aureus	12	10	-
Enterobacter aerogenes	12	10	-
Pseudomonas putida	10	8	-

Table.2 Antibacterial activity of silver nanoparticles of *Calocybe indica*

Bacterial strains	Zone of inhibition (mm)		
	Synthesis of silver nanoparticles in *Calocybe indica*	**Mushroom extract**	**Control**
Klebsiella pneumoniae	10	8	-
Escherichia coli	12	10	-
Staphylococcus aureus	9	8	-
Enterobacter aerogenes	11	9	-
Pseudomonas putida	10	8	-

Table.3 Antibacterial activity of silver nanoparticles of *Pleurotus florida*

Bacterial strains	Zone of inhibition(mm)		
	Synthesis of silver nanoparticles in *Pleurotus florida*	**Mushroom extract**	**Control**
Klebsiella pneumoniae	7	6	-
Escherichia coli	9	8	-
Staphylococcus aureus	9	7	-
Enterobacter aerogenes	8	7	-
Pseudomonas putida	8	7	-

The minimum zone of inhibition was observed in *E.coli* (12mm). The mushroom extract (*Calocybe indica*) showed moderate activity against *E.coli* (10mm). Zone formation was absent in all bacterial strains tested (Table 2).

Kaviya *et al.,* (2011) reported that the silver nanoparticles exhibited good antibacterial activity against both gram negative and gram positive bacteria. But it showed higher antibacterial activity against *E.coli* and *P.aeroginosa* (Gram

negative) than *S.aureus* (Gram positive). Silver nanoparticle undergo an interaction with bacterial cells,and the present study suggested that these showed strong action against *E.coli* (9mm) and *staphylococcus aureus* (7mm). *Enterobacter aerogenes* (7mm) and *Pseudomonas putida* (7mm) were susceptible for mushroom extract (*Pleurotus florida*). In control there was no zone formation in all bacterial strains tested (Table 3 and Fig 3).

The present study clearly indicates that *Pleurotus platypus* showed good antibacterial action against *Enterobacter aerogenes* (18mm) and *E.coli* (16mm), when compared to the control. There was no zone formation in all bacterial strains tested. The mushroom extract (*pleurotus platypus*) showed moderate activity against *Enterobacter aerogenes* (14mm) (Table 4).

Antibiotic sensitivity test of bacteria

The antibiotic sensitivity test using standard antibiotic Streptomycin, Tetracycline and Ampicillin were tested against bacteria studied. The Tetracycline standard antibiotic showed maximum zone of inhibition of *Escherichia coli*(24mm) and *Staphylococcus aureus*(20mm) (Table 5).

FTIR study

Philip (2009) investigated that the FTIR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized using mushroom extract. The silver nanoparticles absorb strongly at 1679, 1539, 1452, 1430 and 1040 cm^{-1}. It is evident from the differences in the absorption that the capping series could be different for gold and silver nanoparticles. In the present study FTIR result revealed that the absorption bands at 3421.14, 2068.19, 1638.39, 692.11which are associated with N-H stretch, primary two bands, amine N-H strecting, Transition metal carbonyls, Unsaturated Nitrogen Compounds-O-No_2, Nitrate. Halogen compounds C-X strecting vibration C-C1 respectively (Table 6 and Fig 1).

UV-visible study

Noginov *et al.*, (2007) Formation of silver and gold nanoparticles from 1mM solution of silver nitrate and auric acid was confirmed by using UV – vis spectral analysis. AgNPs and AuNPs have free electrons, which give rise to a surface plasmon resonance (SPR) absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with the light wave (Nath *et al.*, 2007) surface plasmon resonance spectra for AgNPs and Au NPs were obtained at 451 and 578nm with brown – yellow and pink – red color. In the present study the formation of AgNPs using1mM solution of $AgNO_3$ was confirmed using UV- visible spectral analysis. Metal nanoparticles such as silver have free electrons, which gives rise to surface Plasmon resonance (SPR) absorption band. The characteristic surface Plasmon resonance band of biogenic AgNPs occurs at 300nm. The absorbance scan taken by UV-vis spectrometer showed a sharp Plasmon peak at 435nm confirming the presence of silver (Fig 2).

SEM analysis

Nithya and Ragunathan, (2009) investigated that the SEM micrographs of nanoparticle obtained in the filterate showed that silver nanoparticles are

Table.4 Antibacterial activity of silver nanoparticles on bacterial strains of *Pleurotus platypus*

Bacterial strains	Zone of inhibition (mm)		
	Synthesis of silver nanoparticles in *Pleurotus platypus*	Mushroom extract	Control
Klebsiella pneumoniae	14	12	-
Escherichia coli	16	12	-
Staphylococcus aureus	12	11	-
Enterobacter aerogenes	18	14	-
Pseudomonas putida	14	12	-

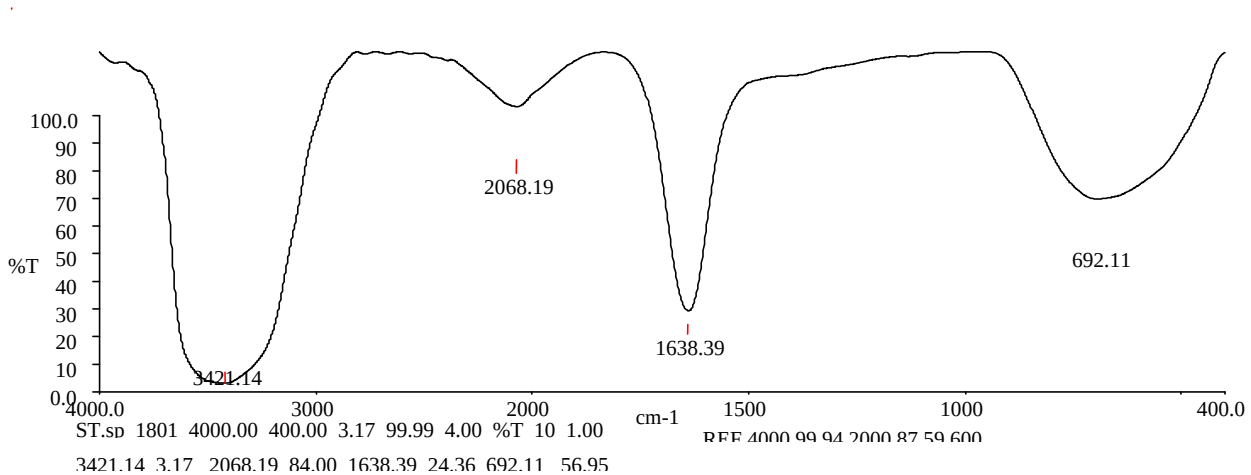
Table.5 Antibiotic sensitivity test on bacteria (Positive control)

Bacterial cultures	Antibiotics(Zone of inhibition diameter in mm)		
	Streptomycin	Tetracycline	Ampicillin
Klebsiellapneumoniae	10	8	8
Escherichia coli	15	24	15
Staphylococcus aureus	10	20	10
Enterobacteraerogenes	8	18	8
Pseudomonas putida	8	8	8

Table.6 Detection of various functional group by FT-IR from silver nanoparticles synthesis of *Pleurotus platypus*

Functional group assignment	Group frequencycm^{-1} of the sample
N-H Stretch, Primary two bands, amine N-H stretching	3421.14
Transition metal carbonyls	2068.19
Unsaturated Nitrogen compounds O-NO_2, Nitrate	1638.39
Halogen compounds C-X stretching vibrations C-Cl	692.46

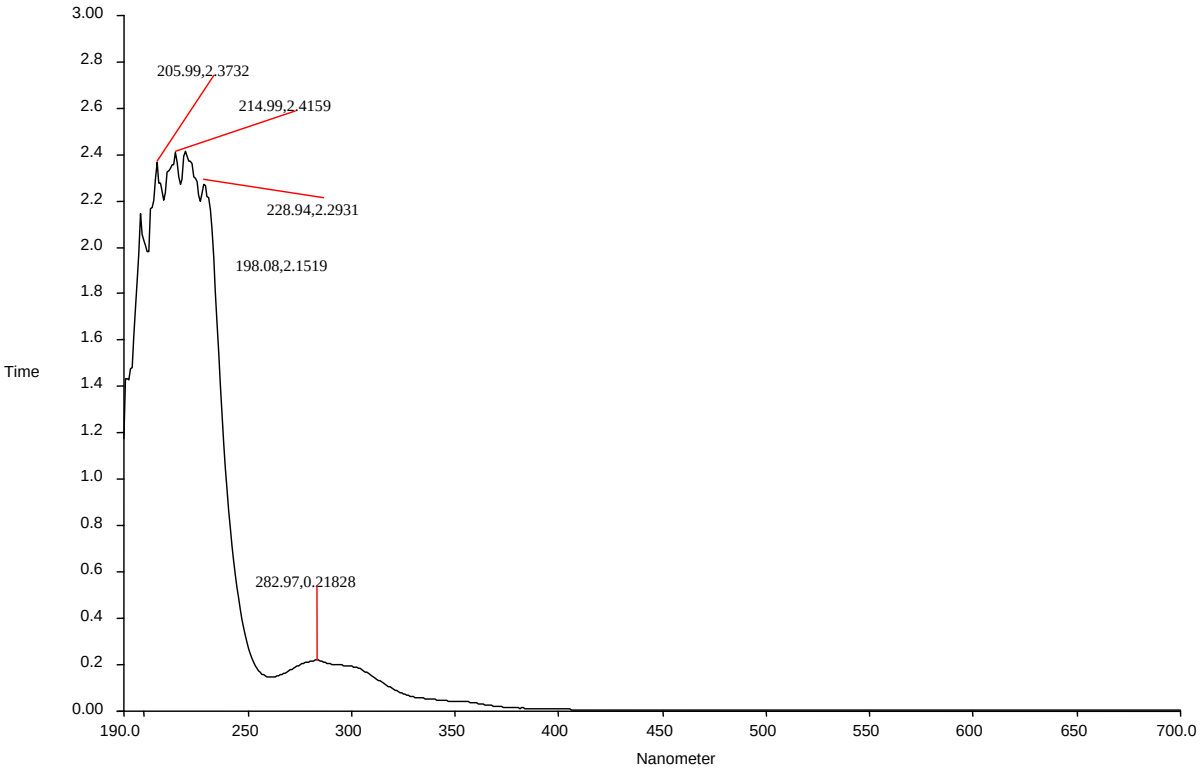
Fig. 1 Detection of various functional group by FT-IR from silver nanoparticle synthesis of *Pleurotus platypus*



spherical shaped, well distributed without aggregation in solution with an average size of about 5 – 50nm. In the present study the SEM micrographs of nanoparticle obtained in the filtrate showed that silver nanoparticles were spherical shaped, well distributed without aggregation in solution with an average size of about 0.56µmto 0.71µm (Fig 3).

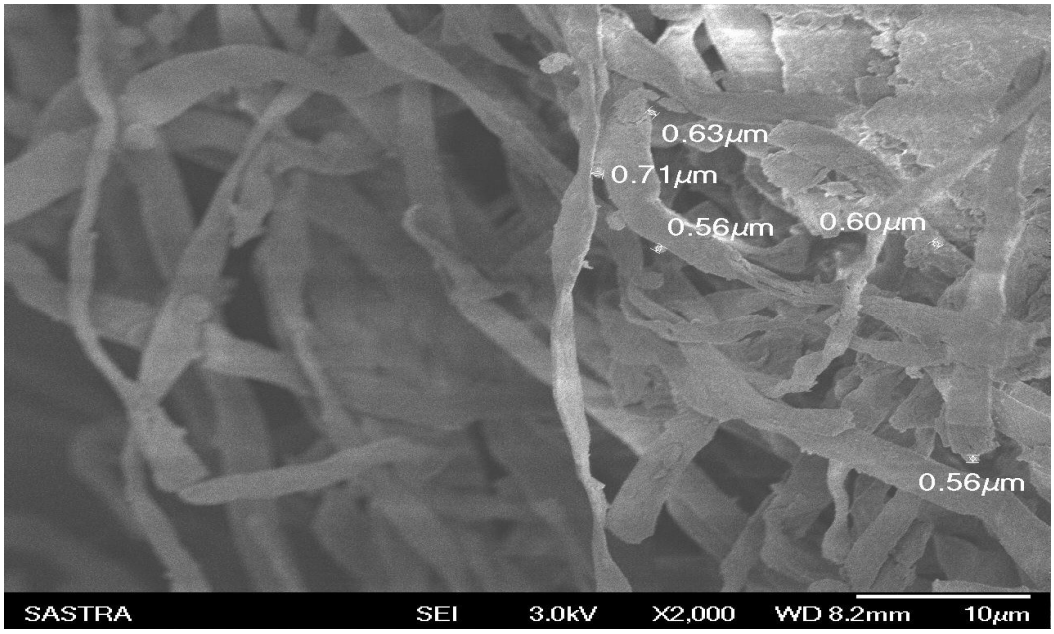
This study concluded that silver nanoparticles play a significant role in the field of biology and medicine. The high bactericidal activity is certainly due to the silver cations released from Ag nanoparticles that act as reservoirs for the Ag^{+} bactericidal agent. Nanotechnology is mainly concerned with synthesis of nanoparticles of variable size. Shape, chemical composition and controlled disparity and their potential use of human benefits. Scanning electron microscopy has provided further insight into the morphology and size detail of the synthesized nanoparticle.

Fig. 2 UV Spectrum analysis from silver nanoparticle synthesis of *Pleurotus Platypus*



Instrument Model: Lambda 35

Fig.3 Pre SEM analysis of silver nanoparticle synthesis of *Pleurotus platypus*



Acknowledgement

The authors are grateful to the Managing Director, Sri Gowri Biotech Research Academy, Thanjavur (Dt), Tamilnadu for their permission to utilized the laboratory facility.

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