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A review on silver nanoparticles synthesis using microorganisms and plants.

Una revisión sobre la síntesis de nanopartículas de plata utilizando microorganismos y plantas.

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ABSTRACT

One of the study fields that has been expanding over the past decade is the silver nanoparticles synthesis using microorganisms and plants. The main reason is that through this method nanoparticles of different size, shape and morphology can be synthesised through an eco-friendly method. The low energy requirements and low cost of biological approaches are their key advantages. It is considered as an alternative to the chemical and physical approach. Obtained bio particles due to its physicochemical nature possesses biologically active properties such as antimicrobial activity. This study shows some of the methods to synthesise silver nanoparticles that are environmentally friendly.

Keywords: plants, microorganisms, silver nanomaterial, eco-friendly synthesis, antibiotics.

RESUMEN

Uno de los campos de estudio que se ha ido expandiendo durante la última década es la síntesis de nanopartículas de plata utilizando microorganismos y plantas. La razón principal es que a través de este método se pueden sintetizar nanopartículas de diferente tamaño, forma y morfología mediante un método ecológico. Los bajos requisitos de energía y el bajo costo de los enfoques biológicos son sus principales ventajas. Se considera una alternativa al enfoque químico y físico. Las biopartículas obtenidas por su naturaleza fisicoquímica poseen propiedades biológicamente activas como la actividad antimicrobiana. Este estudio muestra algunos de los métodos para sintetizar nanopartículas de plata que son amigables con el medio ambiente.

Palabras clave: plantas, microorganismos, nanomaterial de plata, síntesis ecológica, antibióticos.

INTRODUCTION

In recent years, the field of nanoscience has experienced remarkable growth. Nearly all areas of science and human life are greatly impacted by nanotechnology. The nanoparticles have physical, chemical, and biological characteristics that are different from those of the bulk materials. Nanoparticles have different properties (including Physical, chemical, optical, thermal, and magnetic) which are unique compared to their

bulk material counterpart. For example, silver and gold nanoparticles have been demonstrated to be effective in hindering the growth of both Gram-negative and Gram-positive bacteria. [1]

Depending on their size, shape, and distribution, silver nanoparticles can have new or improved properties. AgNPs have been displayed in various research to show antimicrobial properties. Nanoparticles were previously created using physical and chemical processes. To create nanoparticles that are safe and environmentally friendly, the biological method of nanoparticle synthesis is emerging. It is a relatively new practice to include microorganisms like bacteria, fungus, yeasts, and herbal extracts in the synthesis of nanoparticles [2]. Relying upon where the nanostructures or nanoparticles are made, these techniques can be partitioned into two classes as numerous microorganisms are able to provide inorganic materials either intra- or extracellularly [3].

Numerous microscopic organisms including bacteria, fungi and also various plants have shown the ability in metallic nanoparticles synthesis and all possess their own merits and demerits. Time needed for the synthesis, the methods used -extracellular or intracellular, temperature needed for growth and ease of extraction all assume a significant part in biological nanoparticle production. Finding the right biological technique can rely on various factors. Above all, the sort of metal nanoparticle being investigated is of indispensable thought [1].

Silver nanoparticles stick to the membranes and cell walls of bacteria and may even enter inside of the cell. They harm cellular structures and change the signal transduction system. Various research reports instances where silver nanoparticles were used successfully to control pathogenic microbes. Silver nanoparticles can be made primarily through chemical synthesis or biogenic synthesis. Chemical synthesis uses reagents that decrease the silver ions and stabilise the nanoparticles. Because of their toxicity and potential risk to human health and the environment, biogenic synthesis techniques are becoming more popular. These particles make it possible to create nanoparticles with stronger stability, better physicochemical properties, and lower toxicity [2]

Utilising microbes and plants or as the results of their metabolic activities one can embrace the biogenic synthesis of nanoparticles. Biomolecules from the organism used in the synthesis are used to cap these nanoparticles, which can increase activity. It is clean, inexpensive, sustainable, and quite simple. In the usage of nanoparticles, it offers higher biocompatibility. Silver nanoparticles synthesis using fungal and bacterial strains are more practical because it can easily handle and easy to go for genetical modifications. When microbes such as bacteria or fungus added to the solution, there will be releasing of protein biomass and tyrosine and tryptophan which can donate electrons reduce silver ions in the solution. These techniques assist with making stable nanoparticles without the utilisation of reducing agents which are harmful. Because they are easy to handle and more amenable to genetic modification, bacterial and fungal strains can be used to synthesise silver nanoparticles. Instead of reducing chemicals that are harmful, these techniques aid in the production of stable nanoparticles [2].

In case of bacterial synthesis of silver nanoparticles, the bacteria or their by-products are first grown in a suitable medium before being exposed to a silver nitrate (AgNO3) solution. Fungi are used to make silver nanoparticles by first culturing them on agar, then moving them to a fluid medium. The biomass created is hence

moved to water for release of the compounds that demonstrate in the synthesis. The biomass is taken out and then in the filtrate silver nitrate is added after filtration [2].

In recent years, exploration being performed on the green synthesis of metallic nanoparticles utilising plants and plant extracts is rising. A very significant part of utilising plants rather than microbes for production of nanoparticles is the lack of pathogenicity [1]

The review's aim is to analyse the experiments in which AgNPs produced by microbes and plants as reducing and capping agents, also the use of these materials in many fields. The Procedures for synthesis, process optimization techniques, and the significance of capping the nanoparticles are all covered. The literature shows that different fungi and bacteria strains have the potential to be used in biogenic synthesis, enabling the creation of nanoparticles with different properties. By adjusting variables like temperature, pH, the concentration of the silver precursor, the amount of biomass, etc., the synthesis can be optimised.

Silver nanoparticles synthesis using bacteria: Green synthesis of silver nano particle using bacteria carried out by many researchers has been explained in this topic and a brief has been depicted in Table 1.

Lactic acid bacteria (LAB): One of the simplest methods to synthesise silver nanoparticles using lactic acid bacteria is using yoghurt. First step is to isolate the bacteria. For this there is a need to dilute yoghurt samples and these samples then poured in to MRS agar plates (deMan,Rogosa,Sharpe). An anaerobic jar is used to incubate MRS agar plates. The growth of bacteria can be observed after the incubation process. For further analysis they undergo subculture. The isolated bacteria can be identified macroscopically (eg: gram staining), microscopically(observing colony morphology) and different types of biochemical analysis. For the synthesis process the first step is to filter the lactic acid bacterial culture. Whatman no.1 filter paper can be used to filter the culture. This culture filtrate mixes with silver nitrate solution in an Erlenmayer flask which incubated at room temperature.[4]

Colour change, UV-visible spectroscopy and Scanning electron microscopy are used for the confirmation and characterisation of synthesised silver nanoparticles. A UV-visible spectrometer is used for the spectrometric analysis of reduced Ag+ to silver nanoparticles. SEM can be used for evaluating the morphology of silver nanoparticles and also size and shape. [5]. Assessing the effect of temperature on synthesising silver nanoparticles will help to find the ideal temperature. UV-VIS analysis helps to indicate increase in pH level and the reduction rate of silver ions also shows change [6].

Antibacterial Potential: The antibacterial activity of the biosynthesized silver nanoparticles can be done using agar well diffusion method. First step is to prepare Muller Hinton agar. The plates were swabbed with test organisms. Pathogenic microorganisms such as S.aureus, E.coli, Klebsiella pneumoniae and Bacillus species can be used as indicator organisms. Sterilised well cutter uses to punch the well into the agar. Then diluted sample adds to the well. The zone of inhibition can be observed and measured ,after incubation [4 -5].

Bacillus subtilis: To synthesise AgNPs, supernatant was blended in with silver nitrate solution and another one is needed without silver nitrate to be utilised as a control test. After the incubation all the solutions are preserved in dark. If it shows a yellow to brown colour change it confirms the presence of AgNPs. purification is done by centrifugation method and then collected for characterisation. TEM, SEM and X-ray diffraction can be used to characterise the prepared silver nanoparticles based on its morphology, structure and elemental

composition. FTIR spectroscopy can be used to detect the functional groups. UV–Vis spectra absorbance of silver nitrate and cell free supernatant shows the impact of time on the formation of AgNPs [7].

Antimicrobial activity: Antibacterial activity can be tested against numerous strains like Klebsiella pneumoniae, S. aureus, E.coli, Staphylococcus epidermidis, and Candida albicans (yeast) etc. The medium used is Luria Bertani (LB) broth. Along with that there is positive control tube which is the microorganism in LB broth and a negative control one which is LB broth .The MIC not entirely settled after hatching. After MICs analysis, aliquots from all tubes where analysed and if there is no apparent development noticed will be cultivated in Mueller-Hinton Agar plates (MHA) not enhanced with silver nanoparticles and afterward incubates [8].

The MIC will represent the bacteriostatic and fungistatic effects of the silver nanoparticles versus the tested microbial strains and the MLC represents the bactericidal and fungicidal activities . The MIC will address the fungistatic and bacteriostatic impacts of the silver NPs versus the tried microbial strains and the bactericidal and fungicidal exercises activities can be addressed using MLC [8].

Bacillus cereus: Bacillus cereus (Endophytic bacterium) which is confined from the Garcinia xantho chymus used to synthesise silver nanoparticles . By using the reduction method AgNPs can be synthesised. The colour change from pale white to brown shows the presence of silver nanoparticles. Luria Broth medium needs to be prepared and it inoculates with the endophytic bacterium culture . The culture flasks used for culture need to undergo incubation in a shaking condition. After incubating, the collection of bacterial cell pellets can be done through centrifugation method. To dispose of any nutrition medium biomass washes in sterile distilled water adhering to it that there is a chance of interacting with the silver ion [9].

The formation of AgNPs can be confirmed by using UV-vis spectrum. It can be done by observing the colour change. Scanning electron microscope and transmission electron microscope helps in grasping the morphology, and also size and helps to understand how nanoparticles are distributed .On an air-dried carbon coated sample, composition of the nanoparticles can be carried out. EDAX attachment on a scanning electron microscope is used for doing this [9].

Antibacterial potential: Agar well diffusion assay is used to determine the antibacterial activity. E.coli, Salmonella typhi, Pseudomonas aeruginosa, S. aureus, and Klebsiella pneumoniae etc can be used as test organisms. To determine the antibacterial activity, the test organisms need to be grown in a medium of nutrient broth. Using a minute amount of nutrient broth cultures bacterial lawns can be prepared on a nutrient agar plate. The wells need to be cut and adds AgNP solution. After the incubation zone of inhibition can be examined .(9)

Table 1 - Silver nanoparticles synthesis using bacteria and methods employed.

Species	Method	Antimicrobial activity against	Reference
Lactic acid bacteria (LAB)	Reduction method	Bacillus sp.	(5)
		Streptococcus aureus	
	Agar well diffusion method	Streptococcus pyogenes	(4)
		Klebsiella sp.	
		Pseudomonas aeruginosa	(6)
Bacillus subtilis	Reduction method	Staphylococcus aureus (MRSA),	(7)
		Staphylococcus epidermidis,	
		Klebsiella. pneumoniae,	(8)
		Escherichia coli	
		Candida albicans	
Bacillus cereus	Reduction method	E.coli	
		pseudomonas aeruginosa	(9)
	Agar well diffusion method	Salmonella typhi	
		Klebsiella pneumonia	

Silver nanoparticles synthesis using fungi.

Green synthesis of silver nano particle using fungi carried out by many researchers has been explained in this topic and a brief has been depicted in Table 2.

Fusarium oxysporum: The F. oxysporum strain cultivates and maintains in potato dextrose agar (PDA). It is then inoculates in a medium that contains malt and yeast extract and then undergo incubation [10] F. oxysporum previously grown on PDA inoculates in a medium that contains both yeast and malt extract. Consequently, the biomass should be centrifuged, after that it should be washed with sterile water and weighed. Then in a glass Erlenmeyer flask which contains distilled water a little amount of F. oxysporum biomass needs to be added. Using a nylon membrane filter the components of the fungal aqueous extract can be obtained. By adding silver nitrate solution silver ions can be produced. The solutions need to be kept in the dark [10]. Characterization can be done using TEM, SEM and atomic force microscopy.

Antifungal activity assay: Disk diffusion method with some modifications that can be used for assessing the in vitro antifungal activity of the SNPs. Among Candida species the test organisms are C. albicans ATCC 10231[10]. Müller-Hinton agar Petri plates need to be prepared first and subsequently fungal inoculum uniformly spreads onto the plates. Then, at that point, on Whatman No. 1 sterile filter paper discs, an aliquot of SNP colloidal solution was impregnated. The discs were applied to the plates, which then goes through incubation. Aqueous fungal extracts and water are then used as negative controls for antifungal activity.

Aspergillus niger: Aspergillus niger which isolates from the soil used in the synthesis of SNPs. After microscopic confirmation the fungus is grown on potato dextrose agar and Whatman filter papers No.1 is used to harvest the biomass through filtration. The pure cultures of A. niger can be maintained on potato dextrose agar plant [11].

When silver ions adds to the medium of flasks that contains fungal filtrate , the colour changes to dark brown. This indicates the silver ions reduction by fungus to form AgNPs. Silver nanoparticles synthesised extracellularly by the filtrate of the fungus. This fungus secretes an enzyme, which brings about reduction of silver ion thereby forming the silver nanoparticles .This offers benefits over an intracellular process of synthesis from the application perspective, because of the reason that intracellular process require tedious downstream processes for release of nanoparticles from the cell [11 – 12].

The silver nanoparticles can be characterised by Elemental Spectroscopy imaging and Transmission Electron Microscopy. UV-Vis spectroscopy was used to inspect the size and shape of biosynthesized nanoparticles in aqueous solutions. The presence of fungal protein around the silver nanoparticles is shown by Elemental spectroscopy imaging, subsequently increasing their stability in the suspension [11].

Assessment of Antibacterial Activity: The silver nanoparticles show remarkable antibacterial activity against gram-positive (Staphylococcus. aureus) and gram-negative (Escherichia coli) bacteria. S. aureus which shows maximum growth inhibition than that of E.coli [11]

Aspergillus foetidus: Czapek-Dox (CD) is a broth medium used for the growth of Aspergillus foetidus fungal strain. The pH of the medium should be changed prior to autoclaving [13]. The extracellular filtrate of the Aspergillus foetidus strain can synthesise AgNPs from the aqueous solution of silver nitrate. This kind of silver nanoparticles synthesis may be considered as eco-friendly because it is free from any toxic chemicals or organic solvent. The synthesised silver nanoparticles are viewed to be relatively more stable by the synthesis system itself. So, when we consider cost, it tends to be considered a cheaper process than the other processes.

LCF, the fungal extract, is used as a reducing agent. For silver nanoparticles synthesis, AgNO3, mixes with cell filtrate in a flask and incubates in dark. Positive Control and negative control (silver nitrate solution) also run alongside the experimental flask [13].

AgNPs that are synthesised biologically have been broadly utilised in the field of medicine. The silver nanoparticles synthesis has been carried out by using the extracellular filtrate of Aspergillus foetidus MTCC8876. The formation of silver nanoparticles can be recognised by observing the change in colour of the extracellular filtrate and for confirmation UV-Vis spectroscopy can be used. Zetasizer Nano ZS helps in determining the particle size distribution of AgNPs. Atomic force microscopy is used to study the morphology of silver nanoparticles.

Antifungal activity: To examine the antifungal activities against the fungal strains of Aspergillus species such as Aspergillus niger, Aspergillus foetidus, Aspergillus oryzae, F.oxysporum, Aspergillus parasiticus and Aspergillus flavus, the colloidal suspension of silver nanoparticles is used. This is done by using agar well diffusion method. This indicates that it could be considered as a strong antifungal agent which implies its application in the biomedical field [13].

Lecanicillium lecanii: Maltose Yeast Extract Broth is used for the preparation of fungal inoculum. After the incubation period, biomass is filtered .Filtration is used to separate aqueous solution components. The washed fungal biomass takes in a conical Which has sterile Millipore water under shaking condition. By observing pale yellow to brown colour change of reaction mixture the synthesis of nanoparticles can be confirmed [14]. UV-Vis spectrophotometer and scanning electron microscopy can be used for the further nanoparticles synthesis. It will reveal the strong silver plasmon absorption maxima. Scanning electron microscopy can be used to find the surface topography of silver nanoparticles coated cotton fabrics [14].

Antibacterial activity: Antibacterial properties of coated fabrics are useful for the research against pathogenic bacteria such as S.aureus and E.coli. Further treatment includes assessing the improvement in antibacterial activity of nanoparticles that are coated with cotton fabrics with tetracycline, ofloxacin and neofloxin against E. coli, cloxacillin and ofloxacin against S.aureus. Moreover silver nanoparticles with the tested antibiotics coated cotton will show the expanded range of antibacterial action. It implies that the AgNPs govern the antimicrobial activity of the antibiotics and the conceivable use of nano compound combination effect against pathogenic microorganisms which causes wound infections [14].

Table 2-Synthesis of silver nanoparticles using fungi and methods used

Species	Method	Antimicrobial activity against	Reference
Fusarium oxysporum	Reduction method	Candida spp.	
	Disk diffusion method	Cryptococcus spp.	(10)
Aspergillus Niger	Bioreduction method	Staphylococcus. aureus	
		E.coli	(8,12)
Aspergillus foetidus MTCC8876	Bioreduction	Aspergillus niger	(13)
	Agar well diffusion method	Aspergillus flavus Aspergillus foetidus Aspergillus oryzae Aspergillus parasiticus	
Lecanicillium lecanii	Reduction method	Staphylococcus aureus	(14)
		E.coli	

SILVER NANOPARTICLES SYNTHESIS USING PLANTS

Green synthesis of silver nano particle using plants carried out by many researchers has been explained in this topic and a brief has been depicted in Table 3

Aloe Vera plant extract: For the synthesis, there is a need to form a colloidal solution by grounding aloe vera gel. After filtrating the above colloidal solution, it goes for further analysis. In this aloe vera extract AgNO3 solution needs to be added and finally using ammonia solution it is diluted to adjust the pH of the medium. Colour change of orange yellow indicates the formation AgNP which is having spherical shape can be confirmed

when there is a colour change of orange yellow. The biogenic synthesis of AgNP is mainly monitored by a UV-visible spectrophotometer. The progress of the reaction and the appearance of plasmon bands can be monitored at different time intervals. Fourier transform infrared spectroscopy and X-ray powder diffraction (XRD) pattern are also recorded [15]

Antimicrobial activity: Agar disc diffusion assay method can be used for testing the antibacterial activity of the synthesised AgNPs. Strains of gram positive and gram negative bacteria are used for this purpose . Mueller-Hinton agar medium can be used for inoculating AgNP solution and extract stabilised AgNP also the assay control antibiotics. The zone of inhibition formed around the disc will show the antibacterial activity [15].

Neem extract: Firstly, there is a need to prepare neem leaves extract. For this finely cut Neem leaves need to be finely cut and boil in water .Next step is the filtration. Then the extract of Neem leaves is mixed with $AgNO_3$. The Formation of AgNPs can be confirmed by observing the colour change. The effects of various physicochemical parameters were examined by varying the concentration of reactants, pH, temperature and time needed for the reaction. By using an UV/VIS/NIR spectrometer, absorption spectra can be recorded. FTIR helps to study about the biomolecules that are responsible for the reduction of Ag salt [16].

Antimicrobial activity: The synthesis AgNPs can be tested for their antibacterial property against microbes. Bacteria that are obtained from garden soil samples are suitable for this . The bacteria were grown on agar plates then a modest quantity of AgNPs can be added for the study of antibacterial properties and the zone of clearance can be found. The antibacterial activity of AgNPs can be explained due to the degradation of enzymes in bacteria change in the cell membrane permeability [16].

Parthenium leaf extract: First Leaves need to be dried, cut into fine pieces, boiled in sterile distilled water and filtered. Then add it in to silver nitrate solution and next step is centrifugation. Change in colour from water colour to yellowish brown shows the presence of silver nanoparticles. UV vis spectroscopy is used to investigate shape and size controlled of nanoparticles and TEM analysis can be used for further characterisation [17].

Table 3-Silver nanoparticles synthesis using plants and methods used

Species	method used	Antimicrobial activity against	Reference
Aloe vera	Bioreduction Agar disc diffusion method	gram positive and gram negative bacteria	(15)
Neem	Bioreduction	E.coli and S. aureus	(16)
Parthenium	Bioreduction		(17)

CONCLUSION

Nowadays, methods for producing nanoparticles that use chemicals or physical processes are getting increasingly harmful. An option that is more cost-effective and quicker to complete is using microorganisms or green synthesis. Compared to other biological systems like fungus or plants, bacteria are comparatively

inexpensive to produce and have a rapid pace of growth. As the preferred chassis for the close to-term bioproduction of nanoparticles which requires effective synthesis through genetic engineering, they have an edge over plants and fungi due to their ease of manipulation. As an alternative, fungi have the benefit of creating extremely excessive yields of proteins secreted, which may speed up the manufacturing of nanoparticles. Mycelium, which is found in many fungi, has a surface area that is much larger than that of bacteria. The interaction of metal ions with the fungal reducing agent might be supported by this surface area, which would increase the conversion of ions to metallic nanoparticles.

When extracellular nanoparticles are produced by fungi, it is also easier to process them downstream, making the process of extracting nanoparticles from them more effective. Because fungi can be utilised more easily in large-scale reactors than bacteria. scalability—another factor to consider in the case of the commercial production of nanoparticles. They have the advantage of serving as the chassis of choice for long-term development.

Finally, plants have also been found to be nanoparticle producers. The physiology of plants has made a wide range of studies on them possible. Plant extracts, complete plants, and even individual plant components have all been used in the production of nanoparticles. However, many more examples must be found and, particularly in the case of whole plant synthesis, the risks must be carefully evaluated before any industrial relevance can be attributed to the synthesis of nanoparticles by plants.

This paper shows the several potential uses for the biogenic synthesis of silver nanoparticles using microbes such as bacteria and fungi as well as the plant extracts and their antimicrobial potential against pathogenic organisms.

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