Biosynthesis of nanoparticles by microorganisms and their applications

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ABSTRACT

Nonobiotechnology is defined as the study of biological phenomenon at nanosize scale. Generally, Nanotechnology comprises the study of materials that are less than 100 nm in size. Recently, The study of nanosize particles has gained much attention due to their unique size-dependent properties and their various applications. This brief review will discuss the biosynthesis of metal nanoparticles by different microorganisms, Also it will extend to the applications of these nanoparticles, especially in biology and related fields.

Key words: Nanobiotechnology, nanoparticles, applications, microorganisms, biology.

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INTRODUCTION

The unique characteristics of nanomaterials over their macroscaled counter-parts gave them high importance in a lot of valuable applications due to their altered physical and chemical properties [1]. In addition to their special physical and chemical characteristics, they showed unusual optical, photoelectrochemical and electronic properties [2]

The problems with the physical and chemical methods used for the production of nanoparticles such as: short time stability and safety issues can be solved by the use of other biosynthetic methods such as the use of microorganisms. Many organisms can produce either extracellular or intracellular inorganic substances [3]. Magnetite nanoparticles are an example of nanoparticles produced by unicellular magnetotactic bacteria [4]

Nanoparticles are used recently over a wide range in different applications such as the use of metallic nanoparticles especially, silver nanoparticles as potential antimicrobials. This antimicrobial property of metal nanoparticles can be used in many applications such as in water treatment, food processing, textiles industry and in medicine.

Although its great applications, nanotechnology faces many challenges and issues such as the toxic effect of nano-residues on the environment and the health of human beings which is known as nanotoxicology.

NANOPARTICLES PROPERTIES

Materials properties change greatly as they move towards nanoscale level. In case of nanoparticles, the ratio of atoms at the material surface is higher than the total number of atoms at its bulk which results in the unusual properties of the nanomaterials such as their catalytic promotion to reactions and their ability to adsorb other materials. So nanoparticles have large surface which can carry other substances such as proteins and drugs.

Generally it was found that the size decrease of materials towards nanosize scale leads to change in their optical and electronic properties. Due to their size which is similar to most biological structures, Nanoparticles are used recently in medicine for biomedical research and its applications such as drug delivery and as antimicrobials for highly pathogenic microorganisms [5].

BIOSYNTHESIS OF NANOPARTICLES USING BACTERIA

The biosynthesis of metal nanoparticles by prokaryotic bacteria gained large concern among other microorganisms. Gold nanoparticles were first produced by *bacillus subtilis* by reduction of Au^{3+} ions when it was incubated with gold chloride [6]. Also gold nanoparticles were biosynthesized using *Shewanella algae* which showed an ability to reduce Au(III) ions in presence of anaerobic conditions and hydrogen gas [7].

The different nanoparticle sizes may be attributed to the cell growth and the incubation conditions of the metal. Nanocrystalline silver produced by bacteria may be treated thermally in order to give a carboneous nanoparticles with unusual optical characteristics which can be controlled by changing the silver loading factor. These carboneous nanoparticles can be used in many different applications such as their use in thin-film coating [8].

Lactobacillus strains exposed to silver and gold ions showed a great ability to produce silver and gold nanoparticles. *Lactobacillus* can be used also to produce silver and gold nanoparticles alloy when incubated with a mixture of silver and gold ions [9]. Also *Clostridium thermoaceticum* was found to precipitate CdS in the medium from $CdCl_2$ in presence of cysteine hydrochloride [10]. CdS was reported to be precipitated on *Klebsiella aerogenes* cell surface when incubated with Cd^{2+} ions in the growth medium having a particle size of about 20-200 nm. When exposed to cadmium chloride, *E.coli* formed intracellular CdS nanoparticles [11].

It was found that bacteria growth phase affect greatly on the size of the nanoparticles formed. Nanoparticles formed by *E.coli* at stationary phase were larger in size than that synthesized in logarithmic phase by 20 times [12].

BIOSYNTHESIS OF NANOPARTICLES USING YEAST

Eukaryotic yeasts are used largely in biosynthesis of semi-conductor nanoparticles. CdS nanoparticles were formed intracellularly by *Candida glabrata* when it was incubated with Cd^{2+} ions [13]. Intracellular PbS nanocrystals were formed by *Torulopsis* sp. when exposed to Pb^{2+} ions. These nanoparticles which were produced by *Torulopsis* sp. showed maximum absorption at 330 nm after they were extracted by freeze thawing and their size ranged from 2-5 nm.

Recently, Organic substances are used extensively in electronic devices due to their flexibility and easy modification. Chemical methods for production of nanoparticles showed high efficiency in device applications whereas the use of microorganisms nanoparticles for these applications still under research.

Intracellular CdS nanoparticles produced by *Schizosaccharomyces pombe* yeast cells showed perfect diode properties. *Schizosaccharomyces pombe* nanoparticles sizes ranged from of 1-1.5 nm and could be used in manufacture of heterojunctions with poly(p-phenylenevinylene) [14].

Although nanoparticles produced by yeast for many years were secreted inside yeast cells, Recently extracellular nanoparticles have been biosynthesized by the yeast strain, MKY3 which is silver tolerant. These nanoparticles formed in log growth phase when the yeast strain, MKY3 incubated with Ag^{+} ions and their size ranged from 2-5 nm [15].

BIOSYNTHESIS OF NANOPARTICLES USING FUNGI

The use of fungi in biosynthesis of nanoparticles was discovered recently. Fungi are eukaryotes which are characterized by production of large amounts of enzymes. They are relatively more complicated than prokaryotes in terms of genetic manipulation when used in overexpression of enzymes included in nanoparticles biosynthesis.

Fungi were reported to produce both intracellular and extracellular metal nanoparticles. Intracellular gold nanoparticles were formed by *Verticillium* on exposure to 10^{-4} M HAuCl4 solution which was indicated by appearance of purple color in the fungus biomass [16]. These gold nanoparticles can be easily detected in form of resonance by using UV-Visible absorption spectrum at 550 nm wave length. this resonance pattern cannot be detected before introduction of gold ions and in filtrate after the reaction is completed. Transmission electron microscope can be used also as indicator for formation of gold nanoparticles by *Verticillium*. On the low power, Small gold nanoparticles can be detected on the fungus walls whereas, larger particles can be seen inside the cells. Gold nanoparticles with a size ranging from 5-20 nm can be seen on the microscope higher magnification power. Moreover, the biomass diffraction pattern proved the gold nanoparticles crystalline nature. Similarly, intracellular silver nanoparticles were formed by *Verticillium* sp. on exposure to silver ions [17].

The mechanism of gold and silver nanoparticles biosynthesis by *Verticillium* sp. includes the electrostatic interaction between ions and enzymes carboxylate groups at first, followed by reduction of ions by the enzymes which are present in the cell wall of the fungus mycelia resulting in nuclei formation which grow and accumulate more and more forming these nanoparticles. Table (1) shows the biosynthesis of metal nanoparicles by different microorganisms.

Table 1: The biosynthesis of metal nanoparicles by different microorganisms.

Microorganisms	Nanoparticles produced
Bacteria	
Bacillus subtilis	Gold
Pseudomonas stutzeri	Silver
Klebsiella aerogenes	Cadmium sulfide
Escherichia coli	Cadmium sulfide
Desulfobacteriaceae	Zinc sulfide
Magnetospirillium	Magnetite
Rhodococcus	Gold
Chlorella vulgaris	Gold
Yeast	
Torulopsis sp.	Lead sulfide
Candida glabrata	Cadmium sulfide
Schizosaccharomyces pombe	Cadmium sulfide
Fungi	
Verticillium	Gold, silver
Colletotrichum sp.	Gold
Fusarium oxysporum	Gold, silver

ADVANTAGES OF THE BIOLOGICALLY PRODUCED NANOPARTICLES

Biologically produced nanoparticles are characterized by their safe and green biosynthesis avoiding toxic reagents which are used in chemical methods. In addition to this, biological methods used for production of nanoparticles allows recycling of metals contained in the waste streams, for example, The recycling of silver from waste streams which allows its utilization in different purposes due to its limited resources [18]. Moreover, The reducing microorganisms used in the recycling process might be also a waste stream recovered from agricultural activities [19].

Microorganisms biomass could be used in combination with any other reducing agent for nanoparticles production when they lack the ability of nanomaterials biosynthesis. Another important advantage of using microorganisms in nanoparticles biosynthesis is the continuous production process when non toxic concentrations of metals are used, for example, continuous production of silver nanoparticles can be achieved when microorganisms are exposed to non toxic doses of $AgNO_3$ [20].

Nanoparticles produced by chemical methods are found to be aggregated together when subjected to liquid environments [21]. This results in reduction of their high surface area which is accompanied by a high decrease of their antimicrobial and catalytic properties. On the other hand, silver nanoparticles produced by microorganisms showed high stability over long time period. These findings were confirmed when extracellular nanoparticles produced by *Aspergillus fumigates* showed high stability for four months [22]. Furthermore, *Bacillus subtilis* nanoparticles were stable for six months [23].

Another remarkable advantage of biologically produced nanoparticles from microorganisms is that they are proteins in nature which facilitate the functionalization of these nanoparticles with other biomolecules leading to improvement of their antimicrobial properties by enhancing the interactions with the microorganisms [24].

Finally, nanoparticles produced by microorganisms are characterized by their easy separation for the medium by centrifugation [25].

APPLICATIONS OF NANOPARTICLES PRODUCED BY MICROORGANISMS

Due to the various advantages of nanoparticles produced by microorganisms. Recently, they are used in many different applications.

NANOPARTICLES AS ANTIMICROBIAL AGENTS

Silver nanoparticles are characterized by their strong antimicrobial effect so they can be used to control the growth of microorganisms in different applications. It was found that as the size of nanoparticles decrease their specific surface area increase and consequently their antimicrobial effect increase [26]. Also the shape of nanoparticles have a great effect on their antimicrobial properties, for example, triangular nanoparticles showed a higher antimicrobial properties than other shapes [27].

There are different techniques used in determination of the antimicrobial activity of silver nanoparticles produced by microorganisms such as the minimum inhibitory concentration (MIC), disk diffusion, minimal bactericidal concentration (MBC) and well diffusion method and each technique used with a different application, for example, the minimal inhibitory concentration (MIC) used in applications which inhibit the growth of microorganisms whereas the minimal bactericidal concentration used with applications concerned by microbial contaminations treatment.

The antimicrobial effect of silver nanoparticles produced by microorganisms may be affected largely by other factors such as the medium composition and the inoculums size. Three factors are responsible for the antimicrobial properties of the silver nanoparticles, the first one is the direct interaction between the silver nanoparticles and the microbial cells resulting in cell damage [28], secondly, the damage of cell membranes as a result of the released reactive oxygen [29] and finally, the interference of silver ions with the DNA replication and the inhibition of microbial enzymes through the interaction with the thiol groups [30].

It was found that silver nanoparticles produced by microorganisms are coated with their cell parts which may affect the antimicrobial properties of these nanoparticles, for example, the antimicrobial effect of the nanoparticles produced by *lactobacillus fermentum* was found to be attributed to silver ions release while the direct interaction and the reactive oxygen have a neglected effect which decreased the nanoparticles antimicrobial effect due to their coating by parts of the microorganisms [31].

NANOPARTICLES IN DRINKING WATER TREATMENT

Silver is commonly used for the disinfection of drinking water. Silver nanoparticles biosynthesized by *lactobacillus fermentum* showed a great ability to remove viruses such as bacteriophage UZ1 and murine norovirus 1 within 1-3 hours [32].

For continuous disinfection of the drinking water, biologically produced silver nanoparticles were coated on the water filters which showed a high antiviral effect. Immobilization of the biologically produced silver powder in the polyvinylidene fluoride membranes is another method for viruses removal [33]. The slow release of silver ions is the main cause of the antiviral activity which means that the immobilized membranes can be used only for treatment of water on a limited scale and cannot be used for larger applications.

Also it was found that low concentrations of silver nanoparticles produced by *lactobacillus fermentum* improved the solar water disinfection process during the first 3 hours and also inhibited the bacterial growth during water storage. these results were not obtained when solar water disinfection used alone or along with titanium dioxide [34].

NANOPARTICLES APPLICATIONS IN MEDICINE

Due to the antibiotic resistance developed by bacteria and increased occurrence of the hospital acquired infections, the use of silver nanoparticles in medicine has developed greatly [35]. In addition to its broad spectrum antimicrobial effect, silver nanoparticles are considered to be strong anti-inflammatory agents [36] and also help in acceleration of the wound healing process [37]. So silver nanoparticles can be used in various applications such as medical devices and wound dressings.

Combination of biologically produced silver nanoparticles from the fungus *Trichoderma viride* with other antibiotics increased their antimicrobial effect especially with ampicillin which is known as the synergetic effect [38]**.** Moreover, the antifungal effect of fluconazole increased significantly when combined with silver nanoparticles produced by the fungus *Alternaria alternate* [39]**.**

Both the methicillin-resistant *Streptococcus epidermidis* and *Streptococcus pyogenes* showed high sensitivity to silver nanoparticles produced by *Staphylococcus aureus*. Although their various applications in medicine, silver nanoparticles need to be tested before their medical applications due to the safety and toxicity issues.

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