Journal of Biosafety

Volume 13, Number 3, Fall 2020

pISSN 2717-0632, eISSN 2716-9804

Microorganisms as Biologically Safe Sources for the Synthesis of Metal Nanoparticles

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Resived Date: 2021/02/3, Accepted Date: 2021/02/21

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Abstract

Nanoparticles (NPs) are of great importance in the science of nanobiotechnology, which are particles within the size of 1 to 100 nanometers. The most important and widely used substances are metal nanoparticles. Over the last several years, various physical and chemical methods have been used for the synthesis of metal nanoparticles. Despite the merits of these chemical methods, most of them impose severe environmental problems and biological risks, therefore the present study reports a biological route for the synthesis of metal nanoparticles. Biological methods for nanoparticle synthesis using microorganisms have been suggested as a potential alternative to chemical and physical methods. Compared to the chemical methods, the biological methods are clean, non-toxic, safe, low cost, and environmentally acceptable. The goal of these developments is to allow the use of less toxic chemical substances and reduce energy consumption by using simple, fast and safe routes. In this way, not only the size of the nanoparticles but also their shape is controlled and the NPs are collected as an element in the wall (outside or inside) of the microorganism's cell. Natural microorganisms include bacteria, fungi, yeasts, algae, protozoa, and viruses but the importance of bacteria, fungi, yeasts, and algae in the biosynthesis of nanoparticles is greater than that of other microorganisms and in this research, we will talk about it.

Keywords: Nanoparticles, Microorganism, Metal Nanoparticles, Biosynthesis.

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Introduction

"Nano" is a Greek word meaning very small and is the size of 10-9 meters. Nanobiotechnology is an evolving science in the production and use of nanoscale tools in biological systems. If the size is reduced to the nanoscale, there will be significant changes in the physical properties of the compounds (1)(2). NPs have a special application in the treatment of diseases and drug delivery due to their wide range of properties such as availability, environmental compatibility, ability to deliver drugs purposefully, and controlled drug release. Meanwhile, metal NPs have a well-defined surface area and a large number of atoms on their surface. Many metal NPs introduced from different metals such as silver, aluminum, gold, zinc, carbon, titanium, palladium, iron, and copper (3).

As the surface-to-volume ratio in the particle increases, as a result the electronic, optical, thermal, mechanical and chemical reactivity property of the compound change. In other words, increasing the surface to volume ratio in a particle causes the atoms on the surface to be more active than the atoms inside (4). Hence, the properties of the sample change. Cheap, clean, non-toxic, and environmentally friendly biological methods are needed for synthesis of NPs. Organisms are developing for the synthesis of NPs and have many advantages, including the fact that they don't need to use stabilizing compounds to prevent the nanoparticles NPs from agglomeration and becoming microparticles. Synthesis is possible at low temperatures even at room temperature, and they aren't toxic, it's easy to work with microorganisms. They are less expensive and microorganisms can synthesize metal NPs in the size of 1 to 200 nanometers using inexpensive and renewable reducing agents such as lactate or acetate. Microorganisms deposit nanoscale metals inside or outside the cell wall for metabolism and carry out vital processes and energy supply, supply of structural metals, and reduction of toxins in their living environment (5).

The biogenic synthesis of NPs due to the use of low toxicity chemicals and the use of lower temperatures, low pressure in the synthesis is a very interesting alternative production in terms of being environmentally friendly. Using biological resources such as plants and microorganisms or their products, researchers have developed different biogenic principles for the synthesis of nanomaterials. Microbial synthesis of metal NPs can be done intracellularly or extracellularly. Intracellular synthesis of NPs requires additional steps such as ultrasound or reaction with appropriate detergents to release the synthesized NPs. At the same time, extracellular biosynthesis is inexpensive and requires easier processing (6) and it is in the interest of large-scale production of NPs to explore its potential applications. For this reason, many studies have focused on extracellular methods for the synthesis of metal NPs. Also, in the natural environment, microbes produce nanomaterials as part of their metabolism and therefore can be used for a variety of applications. Microbes reproduce quickly.

Therefore, this feature can be used in various aspects. Biological methods, especially using bacteria, various factors such as amino acids, proteins, cofactors, culture medium compounds, etc. are effective factors in the fabrication of NPs. As a result, in this method, NPs with different sizes are produced. The morphology and size of NPs can be controlled by controlling physical and

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chemical factors such as time, salt concentration, temperature, pH, effect time and optimizing its production (7).

Recently, biosynthesis of NPs has attracted scientists' attention because of the necessity to develop new clean, cost-effective and efficient synthesis techniques. In particular, metal oxide NPs are receiving increasing attention in a large variety of applications. I felt the need to collect all the necessary information and key points by having a comprehensive review of most of the articles in this field.

Association mechanism of microorganisms with metals

The reasons for the association of microorganisms with metals include the following matters:

• Metabolism and vital processes and energy supply

• Supply of structural metals

• Reducing the toxins of living environment through the oxide and reducing metal ions and dissolving it in water, depositing metal, escaping by creating volatile gas, settling metal ions by absorbing them in its wall and preventing penetration in this way, they deposit nanoscale metals inside or outside the cell.

The reaction of metal ions and microorganisms, like the oxidation of metals, is exothermic and produces energy. They also store metals within themselves and convert them into other ions and use their energy to meet their needs and energy. Also, microorganisms in their structure require small amounts of metals (8).

Synthesis of nanoparticles by bacteria

Among microorganisms, bacteria have attracted the most attention in the field of nanoparticle synthesis. The ability of bacteria to produce various inorganic NPs is well known, and research in this area has typically focused on the formation of metal NPs and metal oxides/sulfides. Different bacterias have been isolated from different habitats and nutritional modes are used to synthesize metal nanocrystals (1). Although bacteria are known to have the ability to produce various mineral NPs such as metal, calcium, gypsum, and silicon, research in this area typically focuses on the formation of metals and metal sulfides/oxides (Figure 1). Different bacteria from different habitats and food modes have been studied for the synthesis of metal nanocrystals. Some of the earliest reports of mineral particle reductions and accumulations in bacteria date back to the 1960s, when zinc sulfide was found in sulfate-reducing bacterias. Subsequent studies in this field date back to the 1980s, when Beveridge and Murray explained how incubation of gold chloride with *Bacillus subtilis* resulted in the production of 5 to 25 nm octahedral gold NPs in a bacterial cell (9).

Organophosphate compounds secreted by bacteria are believed to play an important role in the formation of these nanostructures. In 2002, Karthikeyan and Beveridge observed the microbial reduction of gold ions by *Pseudomonas aeruginosa*, which resulted in the intracellular accumulation of gold NPs with a diameter of 20 nm (10). Another study showed that the *Rhodopseudomonas* capsule was able to reduce gold ions to gold NPs. When the biomass capsule of *Rhodopseudomonas* was incubated with gold ions at natural pH, gold nanoparticles with a size of 10 to 20 nm were formed. Besides, when the same reaction was performed under acidic pH conditions, triangular gold NPs as large as 500 nm as well as spherical gold NPs were obtained. Some

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bacteria have been reported to form more than one metal NPs and two metal alloys.

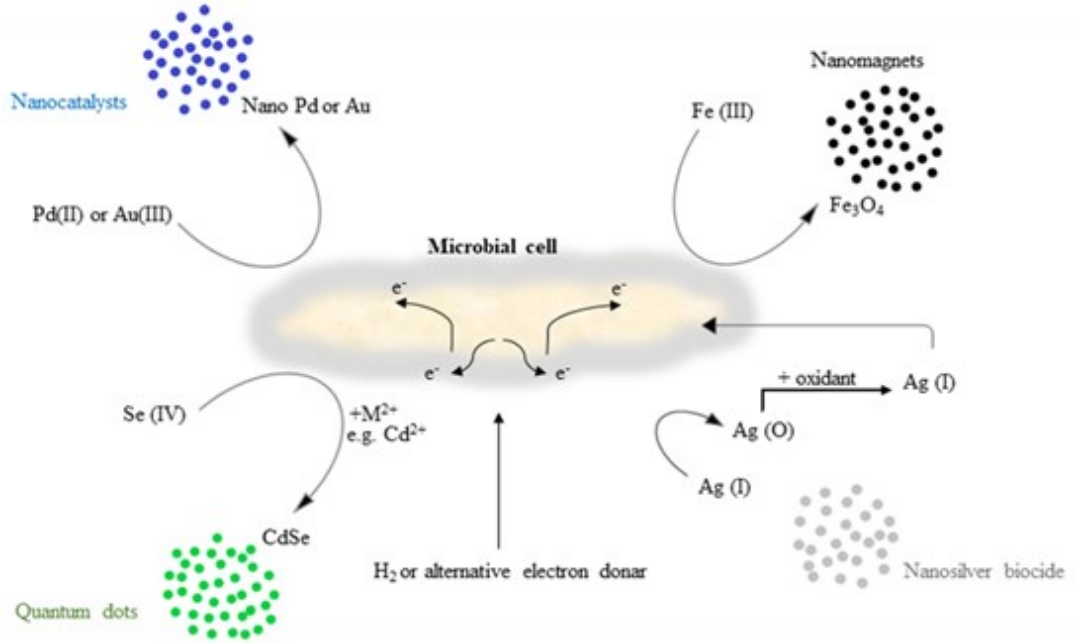

Figure 1. Synthesis of metal nanoparticles by bacteria.

In 2002, Nair and Pardeep synthesized NPs of gold, silver and their alloys using various strains of *Lactobacillus*, bacteria that produce lactic acid (11). It is well known that silver ions are highly toxic to most microbial cells. However, several bacterial strains have been reported to be resistant to silver. *Pseudomonas stutzeri* A259, isolated from a silver mine in Utah (USA) was the first bacterial strain with the reductive potential to form silver crystals. In 2008, Parikh et al. used silver-resistant bacteria to synthesize silver NPs, and there is a direct link between the bacterium's silver-resistance mechanism and the biosynthesis of silver NPs. Three silver-resistant homologous genes (silE, silP, and silS) of *Morganella sp.* were identified. The presence of these genes suggests that the organism has a unique mechanism for protecting against silver ion toxicity, which involves the formation of silver NPs. Similarly, the intracellular production of silver NPs by the highly resistant marine bacterium *Idiomarina sp.* PR58-8 was described in 2012 by Seshadri et al. and strain of *Escherichia coli* AUCAS 11 isolated from plant sediments is also able to detect silver ions more rapidly. Studies of selenium and tellurium respiration have fascinated scientists because these ions are rarely in contact with microbes in their natural environment. Some studies have shown that SeO_3^{-2} reduction may cause periplasmic nitrite reductase in *Thauera selenatis* and *Rhizobium selenitireducens* strain B1, nitrite reductase in *E. coli*, hydrogenase I in *Clostridium pasteurianum*, and arsenate reductase in *Bacillus selenitireducens* or some non-enzymatic reactions (12). Most researches on the biogenesis of selenium NPs are based on anaerobic systems. However, there are few reports in the

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literature on the aerobic formation of these nanostructures by bacteria such as *Bacillus sp*., *P. aeruginosa*, *Enterobacter cloacae*, *P. stutzeri*, and *Pseudo alkaphila* (13). Production of NPs by bacteria compared to eukaryotic microorganisms' easier genetic manipulation is preferred, and the fact is that studies performed on one bacterium can be easily generalized to others. Bacterial cells perform extracellular or intracellular nucleation and growth of NPs through various mechanisms such as enzymatic oxidation, reduction, adsorption and the generalization of peptides or polysaccharides on the cell. Transport of heavy metals in and out through the membranes of microorganisms, post-transfer resistance, and passive mechanisms lead to extracellular deposition, which is characteristic of prokaryotes. Bacteria are microorganisms that are one of the three domains of living organisms. They are prokaryotic organisms, single-celled, and live freely in water or soil or parasitic animals or plants. Due to their abundance in nature, their remarkable capacity to reduce metal ions, as well as their ability to adapt to acute conditions, these bacteria are a good choice as biometric candidates in the production of NPs. Depending on where NPs are formed in bacterial cells, NPs form both inside and outside the cell. Production of intracellular NPs by bacteria, for example, the synthesis of silver NPs such as those produced by *Rhodococcus sp.* Inside the cell and in the cytoplasm, *Vibrio alginolyticus* is produced by the reduction of silver nitrate and *P. stutzeri* AG 259 which are produced in the periplasmic space of bacterial NPs (1). Gold NPs are produced by *Shewanella* algae, an anaerobic, gram-negative bacterium found mainly in marine sediments and in contact with fish. *Brevibacterium casei* produced spherical gold NPs. *E. coli* bacteria synthesize cadmium sulfide nanocrystals intracellularly. Palladium NPs were synthesized by *Desulfovibrio desulfuricans* and *Bacillus benzeovorans* (14). Non-metallic NPs such as selenium were synthesized by *Bacillus licheniformis* JS2, which can inhibit the proliferation and induction of caspase-independent necrosis in human prostate adenocarcinoma cells. The potential of several bacteria for extracellular synthesis of NPs has been studied. Zonooz and Salouti reported the biosynthesis of silver the NPs using *Streptomyces sp*. supernatant as a natural reductant. *Bacillus megaterium* demonstrated the ability to extracellularly synthesize NPs of silver, lead and cadmium. Gold NPs were synthesized by *Pseudomonas denitrificans* (15). *B. subtilis* can synthesize iron oxide NPs extracellularly. Table 1 has shown the other examples of NPs produced by bacteria (5).

Table 1. Summary of some of the reports for production of NPs by bacteria.

Bacteria species	Metal NPs	Location	Size (nm)	Refereces
Serratia nematodiphila	Ag	Extracellular	10-31	(16)
Shewanella oneidensis	U(uranium)	Extracellular	150	(17)
B. casei	Au, Ag	Extracellular	10–50	(18)
Lactobacillus sp.	Au, Ag	Intracellular	20-50	(11)
Acetobacter xylinum	Ag	Extracellular	-	(19)
B. selenitireducens	Te	Extracellular	~10	(12)

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Magnetospirillum Magnetotacticum	Fe_3O_4	Intracellular	47.1	(20)
Shewanella oneidensis	Fe_3O_4	Extracellular	40-50	(21)
D. desulfuricans NCIMB 8307	Pd	Intracellular	~50	(14)
Aquaspirillum	Fe_3O_4	Intracellular	40-50	(22)
Lactobacillus sp.	Ti	Extracellular	40-60	(23)
Lactobacillus sp.	TiO_2	Extracellular	8-35	(24)
P. aeruginosa	Au	Extracellular	15-30	(10)
Thermos scotoductus SA-01	Au	Intracellular Extracellular	-	(25)
Plectonema boryanum UTEX485	Au	Intracellular	10	(26)
Geobacillus sp. strain ID17	Au	Intracellular	5-50	(27)
Deinococcus radiodurans	Au	Intracellular Extracellular	50-60	(28)
Pseudomonas fluorescens	Au	Extracellular	50-70	(15)
E. cloacae, Klebsiella pneumonia, E. coli	Ag	Extracellular	52.5	(13)
Bacillus indicus (MTCC4374)	Ag	Extracellular	2.5–13.3	(29)
B. subtilis EWP-46	Ag	Extracellular	10-20	(9)
Ochrobactrum sp.	Ag	Intracellular Extracellular	38-85	(30)
Nocardiopsis sp. MBRC-1	Ag	Extracellular	30-90	(31)
Brevibacterium frigoritolerans DC2	Ag	Extracellular	50-100	(32)
Aeromonas hydrophila	ZnO	Extracellular	57.72	(33)
Rhodobacter sphaeroides	ZnS	Extracellular	8	(34)
Streptomyces sp. HBUM 171191	$MnSO_4$, $ZnSO_4$	Intracellular	10-20	(35)
Gluconacetobacter xylinus	CdS	Extracellular	30	(36)
Actinobacter sp.	Fe_3O_4	Extracellular	10-40	(37)

Synthesis of nanoparticles by fungi

This is a context that fungi secrete larger amounts of protein, which translates directly into the production of more NPs. Gold NPs were synthesized intracellularly by *Verticillium luteoalbum* (38). Here, by controlling parameters such as pH, temperature, metal concentration, and exposure time, it partially manipulated the formation rate and subsequently the size of NPs. Biological processes with the ability to precisely control the shape of particles are eukaryotic organisms, multicellular, and they have spore production that can be produced both sexually and asexually. These eukaryotic microorganisms have various remarkable characteristics that are well expressed, including the ability to secrete extracellular enzymes, easy operation and growth control, extracellular synthesis of NPs, and having biomolecules in the cell wall which play an important role in the absorption of various metals. Extracellular secretion of microorganisms offers some advantages such as obtaining large amounts in

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relatively pure form and free from other cellular proteins associated with the organism with relatively simple downstream processing (39). It was hypothesized that the proteins, polysaccharides, and organic acids released by the fungus are able to differentiate between different crystalline forms and are also able to grow broad spherical crystals. Das and Marsili studied the role of bacteria and fungi in the synthesis of metal NPs as well as the possible mechanisms. They proposed the synthesis of NPs using microorganisms (Figure 2).

Figure 2. Biomineralization process in nanoparticles synthesis.

According to them, natural processes such as biomineralization may be mimicked to design efficient NPs synthesis techniques. Biomineralization processes use biomolecular patterns that interact with the minerals during their formation, and the results are reflected in the defined shape and size of the NPs. 'Biomineralization' is a term that is used to describe microorganisms for the extraction of precious metals from ore (40). Mukherjee et al. in 2001 used fungal systems to synthesize silver and gold NPs. They observed that for *Verticillium sp.*, intracellular metal ion reduction occurred, resulting in the formation of gold and silver NPs ranging in size from 2 to 20 nm (41). Suster et al. in 2003 also used fungal systems and *actinomycetes* to synthesize silver NPs and gold nanocrystals using intracellular and extracellular processes, respectively. In the case of *Thermomonospora sp.* when exposed to aqueous gold ions, the metal ions are reduced extracellularly, yielding gold NPs with very high

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dispersion properties in the range of 7 to 12 nm. In 2003, Ahmed et al. used *Fusarium oxysporum* to synthesize silver NPs. They reported that when exposed to the *F. oxysporum* fungi, the aqueous silver ions were reduced in solution, resulting in the formation of a very stable silver hydrocele (42). The silver NPs were 5 to 15 nanometers in size and were fixed in solution by proteins secreted by the fungus. Benzol et al. reported in 2004 that the placement of *F. oxysporum* exposed to an aqueous solution of K2ZrF6 leads to the formation of crystalline Zirconium NPs (43). They concluded that *F. oxysporum* is a plant pathogen that is not exposed to such ions during its life cycle, secreting proteins that are capable of hydrolyzing ZrF62-. Ingle et al. in 2009 from *F. solani* isolated used infected onion to synthesize silver NPs. Spherical NPs were synthesized in the size range of 5 to 35 nm. In 2007, Vigneshwaran et al. reported the potential of *A. flavus* for the intracellular production of silver NPs. When worked with hydrate silver ions, silver NPs are synthesized in the cell wall. Maharashtra et al. in 2012 reported the extracellular synthesis of silver NPs using *A. flavus* that were isolated from the soil of Ahar copper mines (44). Filamentous fungi are capable of producing metal NPs and nanostructures by reducing intracellular or extracellular enzymes and through the process of mineralization and biological reduction. *Rhizopus stolonifer* fungi Has been reported to have antibacterial activity due to the synthesis of silver NPs. Biosynthesis of silver NPs by filamentous fungus *Verticillium sp.* has also been reported. Zhang et al. described the use of *Penicillium sp.* fungus for the intracellular production of gold NPs (45). Extracellular synthesis of copper NPs was performed using the dead biomass of *Hypocrea lixii* and *Trichoderma konichiopsis*. Syed and Ahmed reported the quantum biosynthesis of CdTe by *F. oxysporum*. Nickel oxide NPs in the film were produced by *Aspergillus* with an average size of 5.89 nm and nickel oxide NPs inside and outside the cell were also synthesized by spherical *H. lixii* (44). Rajkumar et al. reported the synthesis of TiO2 NPs with possible antibacterial properties from the *A. flavus* (46). The exact mechanism of intracellular or extracellular production of NPs from filamentous fungi has not been fully elucidated. Salvador et al. have proposed a possible mechanism for the intracellular synthesis of NPs, in which the metal binds to the fungal cellular protein by electrostatic interactions. In the next step, metal ions are bio-reduced by enzymes inside the wall, which leads to the accumulation of metal ions and the formation of NPs. The extracellular mechanism of NPs synthesis involves the interaction between metal and enzymes located in the cell wall of filamentous fungi and their subsequent reduction and the formation of NPs. The synthesis has advantages because it doesn't require NPs recovery and lysis of the fungal cell wall. Other examples of NPs produced by fungi are given in Table 2 (5).

Table 2. Summary of some of the reports for production of NPs by fungi.

Fungi species	Metal NPs	Location	Size (nm)	References
Fusarium semitectum	Ag	Extracellular	10-60	(47)
F. oxysporum	Ag	Extracellular	3.4-26.8	(42)
F. oxysporum	TiO_2	Extracellular	6-13	(46)
F. oxysporum	SiO_2	Extracellular	5–15	(46)

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F. oxysporum	ZrO_2	Extracellular	3-11	(43)
Aspergillus clavatus	Ag	Extracellular	10-25	(48)
Candida utilis NCIM 3469	Ag	Extracellular	20-80	(49)
Aspergillus niger	Ag	Extracellular	3-30	(50)
Bipolaris nodulosa	Ag	Extracellular	10-60	(51)
Trichoderma viride	Ag	Extracellular	5-40	(52)
Phoma glomerata	Ag	Extracellular	60-80	(53)
Schizophyllum commune	Ag	Extracellular Intracellular	51-93	(39)
Mucor hiemalis	Ag	Extracellular	5-15	(54)
Penicillium fellutanum	Ag	Extracellular	5-25	(55)
Macrophomina phaseolina	Ag	Extracellular	5-40	(56)
Lecanicillium lecanii	Ag	Extracellular	45-100	(57)
Alternaria alternata	Ag	Extracellular	20-60	(58)
Aspergillus terreus	Mg	Extracellular	48-98	(59)
Aspergillus aculeatus	NiO	Extracellular	5.89	(60)
H. lixii	NiO	Intracellular Extracellular	1.25 3.8	(61)
Aspergillus niger	CeO_2	Extracellular	5-20	(62)
H. Lixii	Cu	Extracellular	24.5	(44)
Candida albicans	ZnO	Extracellular	15-25	(63)
Aspergillus terreus	ZnO	Extracellular	54.8–82.6	(64)
Aspergillus fumigatus TFR-8	ZnO	Extracellular	1.2-6.8 (DLS)	(65)
T. koningiopsis	Cu	Extracellular	87.5	(66)
Colletotrichum sp.	Au	Extracellular	8-40	(67)
Neurospora crassa	Au	Intracellular Extracellular	28-32	(45)
Saccharomyces cerevisiae	Au	Cell wall	15-20	(68)
Rhizipus oryzae	Au	Cell surface	10	(69)
Verticillium luteoalbum	Au	Intracellular	10	(38)
Aureobasidium pullulans	Au	Intracellular	29	(70)
Coriolus versicolor	CdS	Extracellular	25–75	(71)

Synthesize of nanoparticles by yeasts

Yeasts are eukaryotic microorganisms that are classified in the fungus kingdom and about 1500 species have been described in it. There is a new evolution in the use of yeasts to the synthesis of NPs intracellularly and extracellularly through biological pathways. Over the past few decades, yeasts have become increasingly important in nanotechnology due to their valuable NPs production properties, including ease of in vitro control, growth in high temperature, pH and nutrient growth, rapid growth, and production of various enzymes, simple scaling, cost-effectiveness, easy processing, and biomass usage. Several research projects have been performed to obtain the biosynthesis of metal NPs using yeasts (Table 3).

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Table 3. Summary of some of the reports for production of NPs by yeast.

Yeast species	Metal NPs	Location	Size (nm)	References
Candida utilis	Au	Intracellular	-	(41)
Candida glabrata	CdS	Intra and extracellular	20 Å, 29 Å	(72)
Saccharomyces cerevisiae	Sb_2O_3	Intracellular	2-10	(74)
Yarrowia lipolytica NCIM3589	Au	Cell surface	Varying	(75)
Baker's yeast	Au	Extracellular	13	(77)
Candida albicans	Au	Cell-free extract	5	(78)
Yeast cells	Fe_3O_4	Extracellular	-	(79)
Yeast cells	$FePO_4$	Extracellular	-	(80)
Yeast	Zr	-	-	(81)
Yeast	Au/Ag	Extracellular	9-25	(73)
Pichia kudriavzevii	ZnO	Extracellular	~10-61	(82)
Schizosaccharomyces pombe	CdS	Intracellular	1-1.5	(83)
Yeast	CdS	Intracellular	3.6	(84)
Yeast	$Zn_3(PO_4)_2$	Extracellular	10-80 × 80-200	(85)
Rhodotorula mucilaginosa	Cu	Intracellular	10.5	(76)
R. mucilaginosa	Ni/NiO	Extracellular	5.5	(86)
R. mucilaginosa	Ag	Intracellular	11	(87)
Pichia jadinii	Au	Extracellular	<100	(38)
Yeast strain MKY3	Ag	Extracellular	2-5	(88)

Yeasts belonging to the fungi ascomycete class have been shown to have good potential for NPs synthesis. Yeast production is easily controlled in vitro, the rapid growth of yeast species and the use of simple nutrients has several advantages in the mass production of metal NPs (72). The most important principle for the production of NPs by yeast can be attributed to the presence of oxidoreductases and membrane (cytosols) bound quinones, which are pH-sensitive. Increasing the pH inside the yeast leads to the activation of reductases, which reduce metal ions and at the same time and cause the formation of nanoparticles (Figure3).

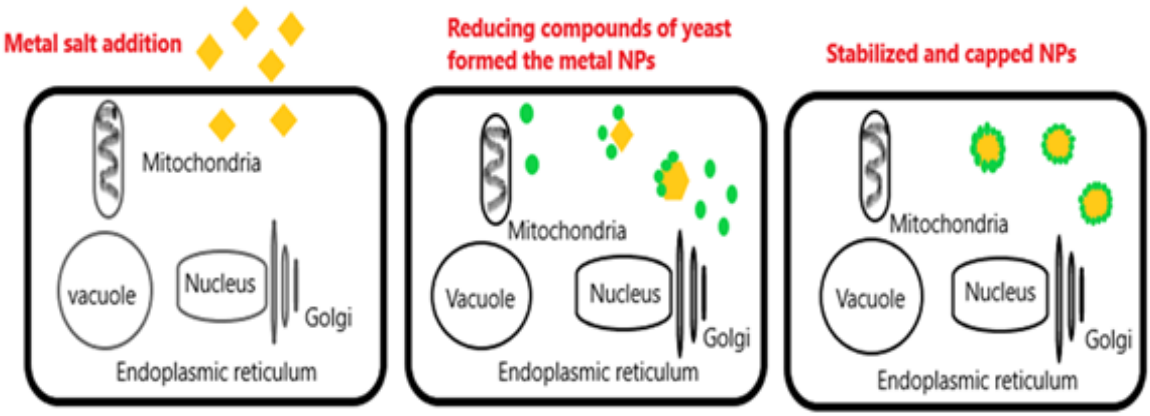

Figure 3. Mechanism of intracellular synthesis of nanoparticles by yeasts.

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The production of stress responses to the presence of metals in the medium nutrient can trigger a metabolic cascade of reactions, leading to the production of phytochelatin synthase and glutathione, which are responsible for reducing inside stress. Both compounds show unique redox and nucleophilic properties due to their ability to bind to cadmium, zinc, silver, selenium, gold, nickel, copper, and other metals leading to metal ion bioreduction are involved in the formation of nanoparticles (73). From these mechanisms, it follows that the main purpose of nanoparticle biosynthesis is to eliminate the toxicity of NPs through cellular mechanisms, and in such cases, the formation of biofilms on the surface of NPs doesn't indicate that the yeast uses biosynthetic NPs for metabolism. The surface of the yeast contains glutamic acid and aspartic acid, which reduce silver ions to silver metals due to sufficient light. In 2015, the yeast species *Candida glabrata* and *Saccharomyces pombe* were considered for the intracellular synthesis of cadmium sulfide, silver, selenium, titanium, and gold NPs (72). Besides, in 2003, the extracellular synthesis of silver and cadmium NPs was investigated. Jaha et al. reported a green, low-cost synthesis of Sb2O3 NPs by Saccharomyces cerevisiae (74). The synthesis of gold and silver NPs by *Candida guilliermondii* and *Saccharomyces cerevisiae* was also described. The ability of marine yeast *Yarrowia lipolytica* NCIM 3589 to produce gold NPs was reported by Agnihotri et al. and the MKY3 yeast strain for the production of silver NPs was reported by Kaushik et al. A green approach using the dead biomass of the yeast *Rhodotorula mucilaginosa* was described as biometric for intracellular production of copper and silver nanoparticles and extracellular production of nickel/nickel oxide magnetic NPs (75). The mechanisms of yeast synthesis by NPs have not yet been elucidated and require further research. El Salvador et al. proposed a natural protocol for the extracellular synthesis of metal NPs using yeasts (76). This mechanism in the interaction between metal cations and amide groups located in the yeast cell wall and subsequent bioreduction is probably due to the presence of extracellular enzymes in the yeast cell wall. These similar proteins act as inhibitors and stabilizers of NPs. The possible mechanism of intracellular production of NPs involves the electrostatic interaction between metal cations and amide groups found in yeast cell wall enzymes, followed by ion bioreduction by enzymes located within the cell wall, which leads to the reduction of metal ions and the formation of NPs. According to numerous available reports, yeasts are considered as a versatile biological material in the manufacture of nanoparticles and have a large share in green nanotechnology (5).

Synthesis of nanoparticles by algae

Algae are single or multicellular organisms, have a nuclear covering and membrane organelle, occupy all the environment to which it provides sufficient moisture and light, and are classified as microalgae and macroalgae (89). They are photosynthetic creatures and can live on the surface of wet rocks, freshwater or seawater, and even snow. Algae can accumulate metals and reduce metal ions, selecting them as a good candida for the synthesis of NPs and also having advantages such as the synthesis of nanoparticles at low temperatures, low toxicity, and easy control. The algae *Tetraselmis kochinensis* was used for the intracellular synthesis of gold NPs with

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dimensions within 5 to 35 nm and were mostly spherical (90). The NPs formed in this way are found more in the cell wall than on the cytoplasmic membrane, indicating that metal ions are reduced by enzymes in the cell wall. In 2010, Marin et al. used four different microalgae called *Chaetoceros calcitrans*, *Chlorella salina*, *Isochrysis galbana*, and *Tetraselmis gracilis* to synthesize silver NPs. The synthesized NPs were also evaluated for their antibacterial potential against human pathogens. There have been numerous reports of NPs synthesis using algae such as *Phaeophyceae*, *Rhodophyceae*, and *Chlorophyceae*. Algae may synthesize NPs from salts of various metals through the functional groups and enzymes in the cell wall, even the oral form of algae is used to make metal NPs. Processing of metal and metal oxide NPs may be performed by several species of algae including *Chlorella vulgaris*, *Spirulina platensis*, *Pithophora oedogonia*, *Focus vesiculosus*, *Sargassum wightii* (91). Freshwater bioassay, an edible red alga *Lemanea fluviatilis*, was used to synthesize gold NPs (92). Abdul Raouf et al. reported the synthesis of silver nanoparticles using the brown seaweed *Padina pavonia*. Fucoidan is a polysaccharide that is secreted from the cell wall of brown seaweed with the ability to synthesize gold NPs (93). *Sargassum muticum* extraction, which is a brown seaweed macroalga, was used in the biosynthesis of ZnO NPs. Nagarajan et al. seaweed extractions of green *Caulerpa peltata*, red Hypnea, and brown *Sargassum myriocystum* were used in the synthesis of ZnO NPs. The results showed that among the three seaweeds, only *S. myriocystum* can stabilize ZnO NPs with a size of 36nm (94). Also, the NPs showed antimicrobial activity against a wide range of bacterial cultures. Figure 4 shows the possible mechanism for the synthesis of metal NPs designed by algae extracts.

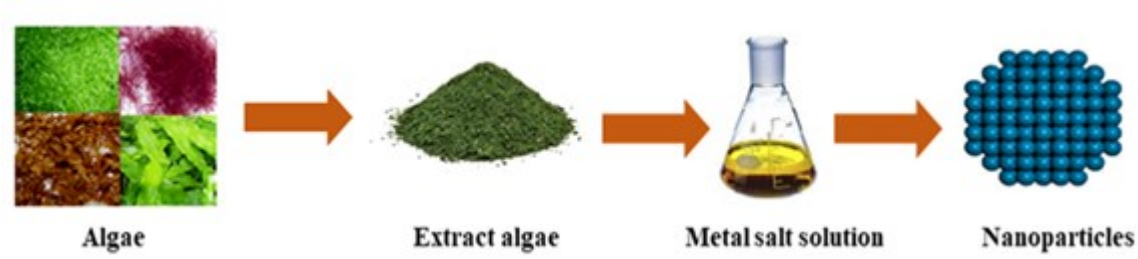

Figure 4. Synthesis of metal nanoparticles by algae.

Concerning other organisms previously studied as nano factors, algae are also important in the biosynthesis of NPs. This study is now called Phyto-nanotechnology. Table 4 shows some samples of nanoparticles synthesized by different species of algae. Algae are members of a diverse group of aquatic photosynthetic organisms and are used in the synthesis of ZnO nanostructures.

Table 4. Summary of some research that has been done to produce nanoparticles.

Algae species	MetalNPs	Location	Size (nm)	References
S. wightii Grevilli	Ag	Extracellular	8-27	(92)
S. muticum	ZnO	Extracellular	30-57	(94)
Sargassum plagiophyllum	AgCl	Extracellular	18-42	(95)

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	(silver chloride)			
Ulva fasciata	Ag	Extracellular	28-41	(96)
Spirogyra varians	Ag	Extracellular	~17.6	(97)
Caulerpa racemose	Ag	Extracellular	5-25	(98)
Cystophora moniliformis	Ag	Extracellular	50-100	(99)
P. pavonica	Ag	Extracellular	54	(89)
P. oedogonia	Ag	Extracellular	25-44	(100)
Parachlorella kessleri	Ag	Extracellular	-	(101)
Chlamydomonas reinhardtii	Ag	Extracellular	5-35	(102)
Chlorella pyrenoidosa	Ag	Extracellular	5-15	(103)
Stoechospermum marginatum	Au	-	18.7-93.7	(104)
S. platensis	Au	Intracellular	5	(91)
Plectonema boryanum	Au	Extracellular	10-6000	(105)
Galaxaura elongata	Au	Extracellular	3.85-77.13	(93)
Ecklonia cava	Au	Extracellular	30 ± 0.25	(106)
T. kochinensis	Au	Intracellular	5-35	(90)
S. muticum	Au	-	5.42 ± 1	(107)
Bifurcaria bifurcate	CuO	Extracellular	5-45	(108)
Amphora-46	Ag	Extracellular	5-70	(109)
Gracilaria dura	Ag	Extracellular	6	(110)
Scendesmus abundans	Ag	Extracellular	59-66	(111)
Chlorococcum humicola	Ag	Intracellular	4-6	(112)
Turbinaria conoides	Au	Extracellular	60	(113)

Conclusion

Bacteria, Fungi, Yeasts and Algae are more important in the biosynthesis of NPs than other microorganisms. Fungi contain extracellular enzymes, obtaining a large scale with economic stability and ease of biomass management is one of the advantages of this method. Genetic manipulation of eukaryotic organisms as a tool of high expression of specific enzyme genes is relatively much more difficult than in prokaryotes. From different biological systems, bacteria are relatively easy to genetically manipulate, while fungi use easy transportation in downstream processing and large-scale production. The large-scale synthesis of NPs using bacteria is interesting because it does not need any hazardous, toxic, and expensive chemical materials for synthesis and stabilization processes. It seems that by optimizing the reaction conditions and selecting the best bacteria, these natural nano factories can be used in the synthesis of stable NPs with well-defined sizes, morphologies, and compositions. The important challenges frequently encountered in the biosynthesis of NPs are to control the shape and size of the particles and to achieve the monodispersity in solution phase. An important challenge is scaling up for production-level the processing. Furthermore, little is known about the mechanistic aspects, and information in this regard is necessary for economic and rational development of NPs biosynthesis.

All shapes are drawn by us and inspired by related shapes in this field.

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"Journal of Biosafety; Volume 13, Number 3, Fall 2020"

مجله ایمنی زیستی

دوره ۱۳، شماره ۳، پائیز ۱۳۹۹

ISSN 2716-9804 الکترونیکی، ISSN 2717-0632 چاپی

میکروارگانیسم‌ها به‌عنوان منابع بیولوژیکی ایمن برای سنتز نانوذرات فلزی

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تاریخ دریافت: ۹۹/۱۱/۱۵، تاریخ پذیرش: ۹۹/۱۲/۰۳

صفحه ۳۲-۱۳

چکیده

نانوذرات اهمیت بسزایی در علم نانوبیوتکنولوژی داشته که ذراتی با اندازه ۱-۱۰۰ نانومتر هستند. طی چند سال گذشته، روش‌های مختلف فیزیکی و شیمیایی برای سنتز نانوذرات فلزی استفاده شده است. بیشتر این روش‌های شیمیایی مشکلات شدید زیست‌محیطی و خطرات بیولوژیکی را تحمیل می‌کنند، بنابراین مطالعه حاضر یک مسیر زیستی برای سنتز نانوذرات فلزی را گزارش می‌کند. روش‌های بیولوژیک به‌عنوان جایگزینی بالقوه نسبت به روش‌های شیمیایی و فیزیکی برای سنتز نانوذرات با استفاده از میکروارگانیسم‌ها پیشنهاد شده است. در مقایسه با روش‌های شیمیایی، روش‌های زیستی تمیز، غیرسمی، ایمن، کم هزینه، ساده و دوستدار محیط زیست هستند. هدف از این تحولات استفاده کمتر از مواد شیمیایی سمی و کاهش مصرف انرژی است. در این روش نه تنها اندازه نانوذرات بلکه شکل آنها نیز کنترل می‌شود و نانوذرات به‌صورت عنصری در جداره‌ی سلول میکروارگانیسم جمع‌آوری می‌شوند. میکروارگانیسم‌های طبیعی شامل باکتری‌ها، قارچ‌ها، مخمرها، جلبک‌ها، پروتوزواها و ویروس‌ها هستند ولی اهمیت باکتری‌ها، قارچ‌ها، مخمرها و جلبک‌ها در سنتز زیستی نانوذرات بیشتر از دیگر ارگانیسم‌ها است که در این پژوهش به آنها می‌پردازیم.

واژه‌های کلیدی: نانوذرات، میکروارگانیسم، نانوذرات فلزی، بیوسنتز.

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