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# Microbial Processes for Synthesizing Nanomaterials

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# Biosynthesis, Characterization and Applications of Gold Nanoparticles

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## Abstract

Nanotechnology is the study of matter at nanolevel. At nanolevel, the physical and chemical properties of the matter changed owing to various applications in various fields. Various metals and metal-oxides were used for the synthesis of nanoparticles (NPs). But among them, gold (Au) plays a precious role. Since the last decade, gold nanoparticles (AuNPs) have its unique features, including electrical, photothermal, and optical characteristics. Various methods were employed for the synthesis of gold nanoparticles, including physical, chemical and biological methods. Among them, biological methods of gold nanoparticle production are gaining popularity because of their ecofriendly methods, cheap, safe, etc. The current book chapter focuses on gold nanoparticles synthesis and their mechanism of formation by using microbes such as bacteria, fungus, actinomycetes, algae, and viruses. In addition to this, synthesized gold nanoparticle was subjected to characterization by using different techniques such as X-ray,

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Fourier transform infrared and UV–visible spectroscopy transmission electron microscopy, diffraction spectroscopy, scanning electron microscopy, atomic force microscopy, X-ray photoelectron spectroscopy, and electron dispersive X-ray. Finally, applications of gold nanoparticles were discussed.

**Keywords**

Gold nanoparticles · Characterization · Nanotechnology · Applications

## 3.1 Introduction

Nanotechnology involves principles of biology, physics, and chemistry for the production of nanoparticles (NPs) with specific purposes (Menon et al. 2017). NPs are characterized as having a size between 10 and 1000 nm. However, due to their comparable sizes to biomolecules and ease of penetrating, it is generally believed that materials smaller than 100 nm are beneficial for applications. Silver (Ag), gold (Au), platinum, palladium, and oxides like titanium oxide and zinc oxide possess special properties like electrical, mechanical, optical, chemical, and magnetic, which have been extensively used for NPs synthesis (Menon et al. 2017). Increased surface area to volume ratio, size, sphere, or rod shaped with special characteristics of nanomaterials with reduced size offers a wide range of biological research opportunities. Nanomaterials can interact with complicated biological systems in novel ways because of their dimensions, which are similar to those of biomolecules. Additionally, nanocarriers interact with proteins both inside and outside of the cell in such a way that cannot change their biological characteristics and activity (Gao et al. 2021). Such easy access to a living cell's inside offers tremendous benefits for basic and clinical research. In the treatment of disease, they aid in enhancing therapeutic efficacy and reducing drug toxicity (Pastorino et al. 2019; Ahmad et al. 2021). In addition to this, NPs have wider applications, including optoelectronics, display devices, catalysis, the fabrication of biological sensors, in the diagnostics involved in disease monitoring (cancer cells), drug discovery, the toxic metals detection, etc (Prasad et al. 2016).

## 3.2 Methods of Nanoparticles Production

Production of NPs by various methodologies has increased, but they were safe, environmentally safe processes that do not depend on using hazardous chemicals. Physical, chemical, and biological were three routes for NPs production fall under either the top-down or bottom-up categories. Size reduction by mechanical method used in the top-down strategy entails gradually disintegrating bulk materials into the nanolevel. Bottom-up approach is based on the assembly of nanoscale atoms or molecules into the molecular structure. Physical or chemical methods refer to top-down, but the bottom-up strategy refers to chemical and biological routes of NPs production (Lombardo et al. 2020). High purity NPs of the desired size have been produced using physical and chemical methods, but these procedures are frequently expensive and are hazardous substances, which is the major drawbacks of physical methods. In the chemical synthesis, some toxic chemical species may end up being adsorbed onto the surface of NPs, causing toxicity when interact with human body.

To overcome these drawbacks, biological processes has paying their attention because they are quick, affordable, and environmentally beneficial. In comparison to chemical or physical processes, green synthesis techniques like biological ones offer a method for synthesizing NPs that is affordable, sustainable, and less abrasive (Prasad et al. 2016, 2018, Srivastava et al. 2021; Kisimba et al. 2023). Biological synthesis additionally provides control over size and form for necessary applications. Many species can create inorganic compounds either intracellularly or extracellularly, as is now widely known (Ghosh et al. 2021). Due to this, a wide variety of natural species, including algae, fungi, bacteria, viruses, and plants, are used for the biological synthesis of NPs. Green synthesis of NPs by algae is considered as "bio-nano factories" due to its unique structure, macroscopic size, and high metal uptake capacity (Aziz et al. 2014, 2015). With the aid of enzymes found in bacteria or plants, the hazardous compounds formed during the synthesis of NPs can be quickly broken down. For instance, the bio-reduction of NPs in fungi is mediated by nitrate reductase (Menon et al. 2017). In reality, respiration processes carried out by bacteria contribute to several metal oxides production (Kim et al. 2018). In anaerobic respiration, microbes transfer electrons from reduced organic to oxidized inorganic molecules, enabling the production of crystal/NPs and bioremediation processes. The ability of the genus *Shewanella* to oxidize organic acids as donors of electrons and reduce inorganic metals as electron acceptors has been well-documented (Harris et al. 2018).

Microorganisms generally have two different effects on the production of minerals. They can alter the solution's makeup to make it more or less supersaturated. Production of organic polymers by microbes is a second way they can affect mineral formation. The most effective environmentally safe green nanofactories for regulating the size of biological NPs are microbes. Plant-based NPs production yields polydisperse NPs due to the presence of phytochemicals (Ahmad et al. 2021; Prasad 2014). These were diverse benefits of microbes over plants in terms of producing NPs. Several microbes are thought to be viable candidates for NPs manufacturing (Priyadarshini et al. 2013). One of the reduced gold (Au) atoms that result from the reduction of an Au(III) ion binds to the cell surface and combines with other reduced Au to form gold nanoparticles (AuNPs). AuNPs acquire special properties due to alteration in localized energy levels and innovative unique features with quantum size effects, including small size, localized surface plasmon resonance, and electronic motion with spatial length scale (Bai et al. 2020). AuNPs synthesized from various microbes yield varied shapes with wider applications in different sectors, including clinical, diagnostics, and treatment of diseases.

## 3.3 Microbial Production of Nanoparticles

Microbial production of NPs has more benefits like easy to handle, ability to grow in in-expensive media, maintenance of safety levels, and potential to adsorb metal ions and reduces them into NPs by the microbial enzymes, due to these advantages microorganisms were used for NPs production (Jadoun et al. 2022; Prasad 2016, 2017, 2019a, b).

Microbes can produce either intracellular or extracellular NPs, depending on the environment. The intracellular mechanism involves particular ions being transported into the negatively charged cell wall, where they interact electrostatically with positive-charged metals to diffuse through the cell wall. The poisonous metals are then changed into nontoxic metal NPs by enzymes found in the cell walls of microorganisms. The extracellular method transforms the metallic ions into metallic NPs with the use of fungi or prokaryotic enzymes like nitrate reductase or hydroquinone, similarly AuNPs were made from *Rhodomonas capsulate*. The bacteria use metal binding, vacuole compartmentalization, and volatilization, or conversion, as detoxifying mechanisms (Menon et al. 2017; Koch et al. 2023).

## 3.4 Microbial Strains for the Production of AuNPs

Gold is one of the famous noble metals. It serves as a heat insulator, and some expensive CDs employ it as a reflective layer, coloring ingredient in cranberry glass, it results in a vivid red color. A multitude of types of gold have been utilized in medicine throughout civilization's history. Rheumatic illnesses like discoid lupus erythematosus, restorative dentistry and a number of inflammatory skin conditions like pemphigus, urticarial and psoriasis have all been treated with gold and gold compounds. Patients with facial nerve palsy and lagophthalmos are treated with gold eyelid implants. The properties of AuNPs are thought to differ from those of bulk materials, as is the case with other NPs. Due to their distinctive optical, electrical, and photothermal capabilities, AuNPs have gained prominence in recent years. They are also resistant to oxidation. Variations in AuNPs' phase and shape can change both their chemical and physical characteristics (Amina and Guo 2020).

Traditionally, physical and chemical processes have been used to create AuNPs. Using these approaches, AuNPs with sizes ranging from 1 to 100 nm and various forms have been produced. Despite the fact that these synthesis techniques have been thoroughly investigated, they have some disadvantages, including the employment of toxic chemicals, strict synthesis conditions, iii) an energy-dependent, costly process, and lower productivity. The mixed form of nanoparticles (NPs) produced by current synthetic techniques necessitates expensive and low-yield purifying by centrifugation. These processes also result in greater sludge and pose environmental risks since they use harmful solvents or additives (Ghosh et al. 2021).

Consequently, there is a growing demand to create sustainable, eco-friendly, nontoxic, and clean synthesis processes. The creation of low-cost, high-yield NPs production, enormous diversity, biological systems has been a focus for biological

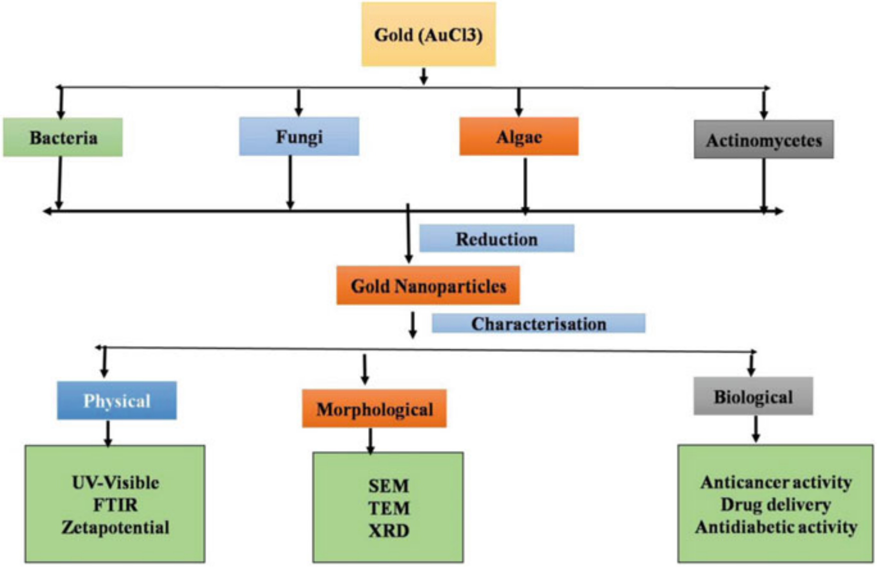

**Fig. 3.1** Gold nanoparticle synthesis by using various microbes and their characterization

NP synthesis (Dikshit et al. 2021). Smaller particles can be produced on a massive scale through biosynthesis. It is significant to mention that biologically created NPs have improved morphological control and increased stability (Kaur et al. 2023). Biological systems with the ability to synthesize NPs include bacteria, fungus, actinomycetes, and plants (Fig. 3.1). Due to their innate potential, bacteria create NPs intracellular and/or extracellular (Pourali et al. 2017). Therefore, a thorough screening of microorganisms that provide extracellular NP biosynthesis is required (Khanna et al. 2023). Microorganisms can be used as potential biofactories to synthesize AuNPs, and this is a relatively new field of study with great potential.

### 3.4.1 Synthesis by Bacterial Strains

Microbes have drawn the most attention in the field of AuNPs production (Table 3.1). First report of AuNPs synthesis was reported by using *Bacillus subtilis* 168, with 5–25 nm NPs within the cell wall. At low concentration, 10–20 nm range spherical AuNPs were formed by *Rhodopseudomonas capsulata*, but at higher concentrations forms nanowires. There are six cyanobacteria that have been identified as producing AuNPs (Shedbalkar et al. 2014). Six cyanobacterial species include *Plectonema*, *Anabaena*, *Calothrix*, and *Leptolyngbya* involved in the synthesis of AuNPs (Pandey et al. 2022). AuNPs and Au core-Ag shell NPs were formed from single-cell protein *Spirulina platensis*. Microbes' amazing capacity to adapt to stressful environmental situations is what leads to the production of reduced metal ions by them (Kulkarni et al. 2015). The inhabitants of gold mines would be

**Table 3.1** Microbial synthesis of gold nanoparticles and their applications

| S. No. | Microorganisms | Size (nm) | Application | Reference |
|---|---|---|---|---|
| Bacteria | | | | |
| 1 | *Paracoccus haeundaensis BC74171* | 20.93 ± 3.46 | Antioxidant activity and antiproliferative effect | Patil et al. (2019) |
| 2 | *Micrococcus yunnanensis* | 53.8 | Antibacterial, anticancer | Jafari et al. (2018) |
| 3 | *Mycobacterium* sp. | 5–55 | Anticancer | Camas et al. (2018) |
| 4 | *Pseudoalteromonas lipolytica* | | Methylene and Congo red decolorization | Kulkarni et al. (2018) |
| 5 | *Bacillus subtilis* | 20–25 | Degradation of methylene blue | Srinath et al. (2018) |
| Actinomycetes | | | | |
| 6 | *Streptomyces griseoruber* | 5–50 | Degradation of methylene blue | Ranjitha et al. (2018) |
| Fungus | | | | |
| 7 | *Trichoderma harzianum* | 32–44 | Antibacterial activity | Tripathi et al. (2018) |
| 8 | *Morchella esculenta* | 16.51 | Antimicrobial activity and cytotoxic activity | Acay (2021) |
| 9 | *Cladosporium sp.* | 5–10 | Photodegradation, in vitro anticancer activity, and in vivo antitumor studies | Munawer et al. (2020) |
| 10 | *Penicillium janthinellum DJP06* | 1–40 | – | Pareek et al. (2020) |
| 11 | *Cladosporium oxysporum AJP03* | 72–21 | Degradation of rhodamine B | Bhargava et al. (2016) |
| 12 | *Rhizopus oryzae* | 16–43 | Hemocompatible activity | Kitching et al. (2016) |
| Algae | | | | |
| 13 | *Spirulina platensis* | 15.60–77.13 | Antiviral activity | El-Sheekh et al. (2022) |
| 14 | *Sargassum cymosum* | 7 and 20 | | Costa et al. (2020) |
| 15 | *Stephanopyxis turris* | 10–30 | | Pytlik et al. (2017) |
| 16 | *Galaxaura elongata* | 3.85–77 | Antibacterial | Abdel-Raouf et al. (2017) |
| 17 | *Cystoseira baccata* | 8.4 | Anticancer | González-Ballesteros et al. (2017) |
| 18 | *Pleurotus ostreatus* | 10–30 | Anticancer and synergistic antimicrobial activity | El Domany et al. (2018) |
| 19 | *Saccharomyces cerevisiae* | | Surface plasmon-enhanced applications | Qu et al. (2018) |

(continued)

**Table 3.1** (continued)

| S. No. | Microorganisms | Size (nm) | Application | Reference |
|---|---|---|---|---|
| 20 | *Phaffia rhodozyma* | 4–7 | Antifungal activity | Rónavári et al. (2018) |
| 21 | *Magnusiomyces ingens* | 20.3–28.3 | Catalyst for nitrophenols reduction | Qu et al. (2018) |
| 22 | *Cystoseira baccata* | 8.4 | Anticancer activity | González-Ballesteros et al. (2017) |
| 23 | *Galaxaura elongata* | 3.8–77.1 | Antibacterial activity | Abdel-Raouf et al. (2017) |

better equipped to withstand the poisonous effects of soluble gold and manufacture AuNPs (Srinath et al. 2018). The color of AuNPs varied with Acinetobacter sp. SW30 incubation due to varied concentrations of gold chloride and different cell densities, indicating change in size and shape. Fe(III) ions are reduced, and the magnetosome vesicles present in *Geobacter* sp., *Magneto spirillum magnetotacticum* undergo dehydration to create magnetite. Iron is stored in vesicles by an intracellular protein called ferritin, which keeps it in a soluble, nontoxic state. The generated NPs exhibit the qualities including great purity, few crystalline flaws, small size, mono-dispersive, and others. The extracellular manufacturing of silver (Ag) and gold (Au) NPs can benefit greatly from the use of thermophilic bacteria. The amount of nanomaterials is produced by these extracellular systems, which reduce the need for further processing of these metals (Jaiswal et al. 2022). Antibacterial drugs that work against Gram$^{+ve}$ or Gram$^{-ve}$ bacteria were synthesized by MDR (multi-drug resistant) microorganisms. It is well-known that Gram$^{-ve}$ bacteria have a very thin peptidoglycan contrasted to Gram$^{+ve}$ bacteria, has thick cell wall and exhibits better antibacterial resistance to drugs; the Gram-negative bacteria have a thin layer of cell wall that is vulnerable to NPs action. Therefore, there is a chance that the AuNPs will also be able to work against Gram$^{+ve}$ bacteria (Menon et al. 2017).

### 3.4.2 Synthesis by Fungal Strains

More fungi have recently been used for research purposes as it has been discovered that they may have a role in the biogenesis of AuNPs. They are commonly utilized because they secrete elevated quantities of enzymes that may be worked on in the lab and have several useful applications. Bacteria and algae, filamentous fungi offer distinct benefits due to their high metal tolerance and capacity for bioaccumulation. They also produce extracellular enzymes, whose manufacture at large scale is simple. Fungal active biomolecules regulate the shape and size distribution of the NPs. They took up the gold ions, which caused the intracellular formation of the AuNPs. When the ultrathin sections of Au-fungal cells were examined, it was discovered that AuNPs had accumulated in the cell vacuoles (Seku et al. 2023).

Because they are easier to cultivate both in the laboratory and on an industrial scale and release a lot of proteins, fungi seem to have more promise for the large-scale production of NPs (Table 3.1). In addition, fungi produce NPs with good monodispersity and specified dimensions. Numerous fungi, including *Fusarium oxysporum* and *Verticillium* sp., have been found to manufacture NPs either intracellular or extracellular (Kumari et al. 2023). Fungal extracts can be used to create gold nanoplates. *Pichia jadinii* and *Yarrowia lipolytica*, two yeasts that have previously been demonstrated to have a good potential for producing AuNPs, are currently being specifically explored for the purpose of engineering AuNPs (Punia et al. 2023).

### 3.4.3 Synthesis by Actinomycete

Actinomycetes are prokaryotes and are easily genetically manipulated to produce NPs of greater size and polydispersed distribution. The prokaryotes' characteristics resemble those of bacteria, but the actinomycetes' similarities to fungi are more striking (mycobacteria and coryneform). They are currently employed in nanotechnology because of their capacity to generate secondary metabolites that resemble antibiotics (Menon et al. 2017). Actinomycetes employ extracellular and intracellular routes for NPs synthesis. AuNPs were synthesized from *Nocardia farcinica*, *Streptomyces viridogens*, *Rhodococcus* species, *Streptomyces hygroscopicus*, and *Thermo actinomycete* species. The intracellular reduction of Au ions lowered AuNPs on the cell wall and membrane rather than in the cytoplasm. A combination of enzymes released from the cell wall and membrane initiated the reduction of AuNPs, and proteins involved in stabilization (Alsaiari et al. 2023).

### 3.4.4 Synthesis Using Strains of Algae

Algae are photoautotrophic, eukaryotic, aquatic, and oxygenic microorganism and can collect heavy metals. Researchers are working to develop more environmentally friendly methods for creating nanoparticles (Table 3.1). This is a benefit of using algae as a plentiful source of raw materials (Babu and Tirkey 2023). The fucoidans are the polysaccharide that marine brown algae cell walls secrete has shown to have several uses in a variety of sectors, including the anticoagulant, anti-inflammatory, antiviral, and even anticancer. They are also utilized as whitening or antiaging agents in the cosmetics industry. These fucoidans can be used to successfully synthesize AuNPs as an alternative to chemical processes as nanophytomedicine (Rathod and Arunkumar 2023). Due to its capacity to absorb heavy metals, brown algae have been utilized more than other species. Their intricate cell wall, which is abundant in mucilaginous polysaccharides, addresses the absorbable nature of heavy metals clearly. Additionally, it has functional groups, such as carboxyl groups, that are important for absorption.

## 3.5 Gold Nanoalloys

Clusters made of two or more metallic elements are known as nanoalloys. Compared with equivalent individual metal NPs, gold nanoalloys have distinctive and frequently improved electrical, optical, catalytic, and magnetic properties. Two metals can be combined naturally or artificially using various production techniques. When compared with monometallic particles, solitary bimetallic nanoparticles have superior physical stability and distinguishing characteristics. Due of their numerous intriguing applications, bimetallic NP synthesis is attracting a lot of attention (Ferrando 2022).

It was discovered that *Fusarium oxysporum* could produce 8–14 nm Au–Ag nanoalloy and that the fungus's NADH-dependent protein controlled the process (Salam et al. 2023). Spirulina platensis synthesizes Ag–Au alloy with 17–25 nm (Khanna et al. 2023). *Neurospora crassa* forms Au–Ag bimetallic alloy on the outer cell wall, when exposed to the ionic solutions of gold and silver (Mohammadi et al. 2023). Changes in the ionic concentration of the solution result in the formation of 3–90 nm of Au–Ag alloy (bimetallic NPs) (Mohammadi et al. 2023).

## 3.6 Gold Nanoconjugates

AuNPs are combined with a variety of biomolecules, including antibodies, proteins, peptides, DNA, and pharmaceuticals, to create gold nanoconjugates. The resulting conjugate displays the selectivity and specificity of NPs as well as their thermal, optical, imaging, and carrier properties. Smaller-sized AuNPs are desirable for immobilizing proteins and enzymes, and these nanoconjugates offer various benefits in biocatalysis, biosensing, and medicine. NPs have a great capacity for enzyme loading due to their high surface area to volume ratio (Oliveira et al. 2023).

## 3.7 Gold Nanoparticles Properties and Characterization

AuNPs display remarkable features that set them apart from metallic gold, indicating the enormous potential for use in medicine. These characteristics include: (1) outstanding optical and electrical properties, (2) facile surface functionalization, (3) excellent chemical and mechanochemical stability, (4) surface plasmon resonance effect, (5) distinct catalytic activity, and (6) biocompatibility. Additionally, the AuNPs have outstanding size dispersion properties, a controlled shape, and resistance to oxidation. Because of their greater surface area to volume ratio, AuNPs with diameters less than 10 nm display entirely distinct physiochemical/thermodynamic characteristics. The stability, optical, and electrical qualities are very crucial since they give AuNPs the rest of their other properties (Amina and Guo 2020).

### 3.7.1 Stability

Although AuNPs have great mechanical and chemical durability, nanostructure aggregation in a surfactant-free reaction is a frequent event that makes it challenging to study their properties and use them. AuNPs can be stabilized by chemical substances such as citrate, ascorbic acid, etc. In addition, the surface of AuNPs can be altered by thiol compounds, cysteine, or polyvinyl alcohol coating. It has been demonstrated that storing AuNPs in the dark at 4°C will increase their stability (Ielo et al. 2021).

### 3.7.2 Optical and Electronic Properties

Vibrant colors are produced by AuNPs through interactions with visible light. The surroundings, size, and physical characteristics of AuNPs have a significant impact on how they interact with light. These distinct optical-electronic characteristics have recently been used in medication administration, therapies, organic photovoltaics, sensory probes, electrical conductors, and catalysis. By altering the size, shape, surface chemistry, or aggregation state, AuNPs' optical and electrical properties can be tailored for use in applications, including drug delivery and targeted cancer imaging (Ielo et al. 2021).

### 3.7.3 Characterization

Numerous methods have been used to characterize gold nanoalloys and AuNPs produced by microbes. However, a variety of methodologies are required for characterization rather than just one methodology. For the primary sample, Ultraviolet–visible spectroscopy is employed for the detection of AuNPs being produced by microbial AuNPs synthesis. *Thermomonospora*, *Rhodopseudomonas capsulata*, *Sclerotium rolfsii*, and *extremophilic* yeasts are a few examples where a peak in the region of 500–550 nm clearly shows the existence of AuNPs (Noah 2019). Fourier Transform Infrared Spectroscopy analysis is used to find the functional groups involved in the reduction of Au ions. X-ray diffraction spectroscopy (XRD) is used to analyze the AuNPs' crystal structure, phase composition, and mean size. The atomic analysis, energy dispersive spectrum (EDS), and energy dispersive X-ray spectroscopy (EDX) can all be used to characterize the electronic state and chemical composition of materials. Characterization of AuNPs is produced by *Escherichia coli*, *Yarrowia lipolytica*, *Rhizopus oryzae*, and *Rhodopseudomonas capsulate* using EDS and EDX (Noah 2019).

Transmission electron microscopy (TEM) is used to map and image NPs in order to better understand their shape. Lattice fringes of AuNPs can be seen using high resonance transmission electron microscopy (HR-TEM) to confirm that its nature is crystalline. Scanning electron microscopy (SEM) is an imaging method that has been utilized to characterize AuNPs. Using HR-TEM, the particle size of *Spirulina* *platensis* was identified as 5 nm with spherical shape (Lasita et al. 2022). According to TEM analysis, the size of *G. stearothermophilus* AuNPs were found to be 11, 12–14, and 5–8 nm sized particles (Molinaro et al. 2022), AUNPs of *P. denitrificans* were found to be 25–30 nm (Bharti et al. 2022). The majority of the AuNPs created by the *Enterobacteriaceae* family of bacteria were spherically shaped (Yassin and Subhi 2022), whereas triangles were produced by the *Bacillaceae* family (Donga and Chanda 2022) and spherical and blunt triangles by the *Pseudomonadaceae* family (Thipe et al. 2022). Using the FE-TEM (field enhanced transmission electron microscope), the *Planococcaceae* family member *Sphingomonas koreensis* created sphere-shaped AuNPs (Suresh et al. 2022). In addition to the techniques outlined above, resonance Raman scattering and cyclic voltammetry have also been utilized for the characterization of gold nanoconjugates. Gold nanoconjugates are also characterized using X-ray electron spectroscopy and thermogravimetric analysis (TGA).

## 3.8 Mechanism Gold Nanoparticles Synthesis by Microbes

A number of bacteria produce AuNPs by the reduction of $Au^{3+}$ ions, but it is hypothesized that the microbial-secreted enzymes majorly involved the inbio-reduction of metal ions results in NPs synthesis. Hydrogenase, and nitrate reductase-mediated synthesis of AuNPs occurs. By understanding the metabolic processes that result in the bio mineralization of gold, a reasonable strategy for producing AuNPs can be created (34).

The synthesis of intracellular AuNPs was discovered to be influenced by physicochemical factors including temperature, pH, and substrate concentration (Banik et al. 2022). According to reports, these factors and the microorganisms' development circumstances can be changed to affect the morphology of AuNPs. By further adjusting these characteristics, monodispersity can be attained. AuNPs were stabilized by proteins and the amino acid residues (cysteine, tyrosine, and tryptophan). Proteins with free amino or cysteine groups can attach to the AuNPs and stabilize them. Tyrosine can occasionally connect to the surface of gold via amine groups and diminish silver ions at high pH, forming nanostructures with an Au core and an Ag shell. At basic pH, tryptophan has also been demonstrated to create metal NPs. By taking an electron from a transitory tryptophyl radical created by the conversion of a tryptophan residue contained in the peptide, a metal ion creates nanoparticles (NPs). Additionally, it was stated that certain proteins had an impact on the capping and stabilization of AuNPs (Banik et al. 2022).

Precipitation of AuNPs in *B. subtilis* 168 cells, while the cells were incubated with $Au^{3+}$ ions, was described in the first mechanistic approach for the bacterial manufacture of AuNPs (Salam et al. 2023). *Shewanella* reduces $Au^{3+}$ ions in the presence of hydrogen gas yielding 10–20 nm AuNPs in anaerobic conditions (Singh et al. 2022). *Desulfovibrio desulfuricans* and *E. coli* reduce $Au^{3+}$ ions and accumulate AuNPs by serving (H2) gas as electron donor and periplasmic hydrogenases involved in bioreduction (Varia et al. 2014). *Plectonema boryanum* UTEX 485, cubic AuNPs precipitate at temperatures between 25 and 100°C for up to a month and 200°C for a single day (Nitnavare et al. 2022). This may be caused by how cyanobacteria and aqueous gold chloride interact, which at first encourages the precipitation of NPs of amorphous gold.

In *Trichothecium* sp., different growth conditions like static and shaking were used to regulate the synthesis of AuNPs. AuNPs were synthesized extracellularly and intracellularly under shaking and static conditions, respectively (Kar 2022). Additionally, mushrooms produce proteins and reduce agents that aid in the stability of NPs produced extracellularly. By regulating the activities and cellular growth circumstances in yeast strains, AuNPs can be synthesized under controlled conditions. The bioreduction of $Au^{3+}$ ions to produce AuNPs is thought to be carried out by species-specific NADH reliant reductases in *Fusarium oxysporum* and *Rhodopseudomonas capsulata* (Noah 2019). The precise mechanism underlying the $Au^{3+}$ ion reduction dependent on NADH is unknown. An innovative fungal enzyme-based invitro technique for nanomaterial production has now become available for the first time. Electrostatic interactions between the cell wall of *Verticillium* with lysine residues in enzymes $Au^{3+}$ ions were trapped on the surface of fungal mycelia surface. As a result, the bioreduction of $Au^{3+}$ ions by enzymes caused metal atoms to aggregate and the creation of AuNPs (Banik et al. 2022).

The proteins and enzymes on the cell surface trap and reduce Au+ ions to create nuclei, which then go through crystal development to form aggregates (size 12,870 nm) of AuNPs at normal pH (2–3.5). Reduction and binding of positive amino and sulfhydryl groups and negative carboxylic groups in proteins mediate the binding of gold ions. A persistent extracellular glycosylated laccase derivative was discovered to be necessary for the formation of extracellular AuNPs in growth media. Enzymes have been demonstrated to be crucial for the stability of NPs and the reduction of metal ions in actinomycete *Thermomonospora*, enabling the effective generation of monodispersed AuNPs (Banik et al. 2022).

## 3.9 Applications of Gold Nanoparticles Produced by Microbes

There are just two papers that indicate AuNPs made by microorganisms that can be employed in medical applications. The AuNPs that *Candida albicans* generate have their potential for detecting whether liver cancer has been researched (116). It has been used to explore the cytotoxic effects of AuNPs produced by *Penicillium brevicompactum* against mouse mayo blast carcinoma C2C12 cells (107).

Nearly every human life is now affected by cancer, and scientists are striving to treat it with gold and silver nanoparticles. The anticancer nanoparticles have been standardized to combat different types of human cancer such as cancerous cells in the prostate, colon, lungs, heart, and breast (Cheng et al. 2021). NPs has the capability for adsorptive behavior and electron transport used in biosensors (Mostafavi et al. 2022). Different kinds of harmful metals from the environment have been detected using nanoparticles (Mitra et al. 2022). Copper necessary for physiological functions but at higher levels induces Wilson’s disorder (Garza et al. 2022).

The usage of AuNPs at the cathodes, along with the quantized charging effect and no external power source, has recently been discovered to be a tremendous assistance in catalyzing the production of hydrogen via microbial fuel cells (MFCS) (Chen et al. 2022).

*Trichoderma* sp. was used to create spherical AuNPs with strong antibacterial activity (Soliman et al. 2022). The experiment for the manufacture of AuNPs with the fungus *Penicillium oxalicum* had shown that at pH levels of 8 and 12, the average particle size determined by TEM and DLS analyses was close to 6 and 4 nm. It was observed that TEM data showed smaller particle sizes than DLS analyses, which may be caused by the NPs tendency to aggregate. They discovered that pH levels between 8 and 12 produced the greatest outcomes, and they used the DLS analysis to confirm particle size and distribution.

The TEM study had shown that the morphologies of the nanoparticles produced by the algal (Galaxaura elongata)-mediated process included spherical (predominantly), rod, triangular or truncated triangular, and even hexagonal (Heinemann and Dias 2022). Although the FTIR results supported the formation of a coat that protects the particle from agglomeration is facilitated by the presence of carbonyl stretch and N-H stretch, which have a stronger capacity to connect with the metal nanoparticle. The zeta potential also verified the particles' stability and surface charge. The sample was a Prasiola crisp, a freshwater epilithic green alga, with dimensions of 5–25 nm and a spherical shape as determined by TEM examination (Menon et al. 2017).

When compared with the TEM investigation, which showed that the *Sargassum swartzii* yields AuNPs in the range of 20–60 nm, DLS measurements showed that the particle size is significantly greater (Costa et al. 2020). The brown alga *Stoechospermum marginatum* formed AuNPs (Murugesan et al. 2017). The minimal size of the NPs in *Thermomonospora* sp. is 8 nm. TEM analysis showed the *Botrytis cinerea's* generated AuNPs size and form (Chowdhury et al. 2022). *Yarrowia lipolytica*, a member of the *Dipodascaceae* family, was used in the manufacture of AuNPs (Kolhe et al. 2022). *Candida guilliermondii* produced spherical particles with sizes between 50 and 70 nm with hexagonal and triangular shapes (Umamaheswari and Abirami 2023). Additionally, the Dipodascaceae family member *Magnusiomyces ingens LH-F1* created nanoparticles with geometries like spheres, triangles, and hexagons that ranged in size from 9.8 to 80.1 nm (Skrotska et al. 2022).

AuNPs has wider applications in various fields as antibacterial agents, antiproliferative agents, dye degradation, etc. The applications of AuNPs were listed in Table 3.1.

*Paracoccus haeundaensis BC74171* AuNPs *were with* 20.93 ± 3.46 nm *possess* antioxidant activity and antiproliferative activity (Patil et al. 2019). *Micrococcus yunnanensis* AuNPs were with 53.8 nm possess antibacterial and anticancer (Jafari et al. 2018). Similarly, anticancer activity of AuNPs was reported by Camas et al. (2018) by using *Mycobacterium* sp., González-Ballesteros et al. (2017) by using *Cystoseira baccata*, El Domany et al. (2018) by using *Pleurotus ostreatus*, González-Ballesteros et al. (2017) by using *Cystoseira baccata*, Abdel-Raouf et al. (2017) by using *Galaxaura elongate*, and Munawer et al. (2020) by using

*Cladosporium* sp. Antibacterial activity of AuNPs was reported by Tripathi et al. (2018) by using *Trichoderma harzianum*, Acay (2021), Abdel-Raouf et al. (2017) by using *Galaxaura elongate*, and Acay (2021) by using *Morchella esculenta*. Dye degradation activity of AuNPs was reported by Kulkarni et al. (2018) by using *Pseudoalteromonas lipolytica*, Srinath et al. (2018) by using *Bacillus subtilis*, and Ranjitha et al. (2018) by using *Streptomyces griseoruber*.

## 3.10 Future Prospects

Microbes were abundant in the environment; hence, various microorganisms were screened for the production of AuNPs. *Glidobacteria*, *beta epsilon*, and *zeta proteobacteria* members have were not reported to synthesize NPs; therefore, groups should be investigated for AuNP production. Monodispersed AuNPs with required size was trustworthy objective for future research for AuNPs produced by microbes. The clarification of the mechanism of AuNPs produced by microbes is one of the difficult problems in nanotechnology. Additionally, a proteomic technique has to be applied to investigate the precise process of AuNP formation. It is necessary to conduct a differential proteome study of the AuNPs production process. The synthesis of proteins and biomolecules that mediate the creation and characterization of AuNPs should also be the subject of research. Studies should concentrate on AuNP stability and methods to stop AuNP aggregation. In addition to their function in synthesis, these proteins are frequently claimed to stabilize the NPs. It is necessary to find newer stabilizing agents for NPs.

## 3.11 Conclusion

Microbes are abundant in the environment, making it crucial to screen each prospective one. It is also vital to take into account the compounds that play a role in how these microbes produce nanoparticles. Consequently, these bimolecular molecules can be characterized physiochemically and via purification. NPs aggregation is another crucial element that needs to be closely monitored because the outcomes of aggregated particles can vary. As a result, it is possible to incorporate novel stabilizing agents into the synthesis to stabilize these nanoparticles. The developing field of bio-nanotechnology has opened up numerous avenues for the development of unique products that can benefit people. As a result, additional study has been conducted to explain how these particles are created and how they may be utilized to cure rare disorders, including all the various cancers. Hence limiting the use of nonbiodegradable plastics and reducing the infiltration of bacteria and dangerous microbes. Additionally, they can function as nano-sensors to help identify harmful metals or food spoilage. These are only a few potential future uses for green synthesis to produce nanoparticles.

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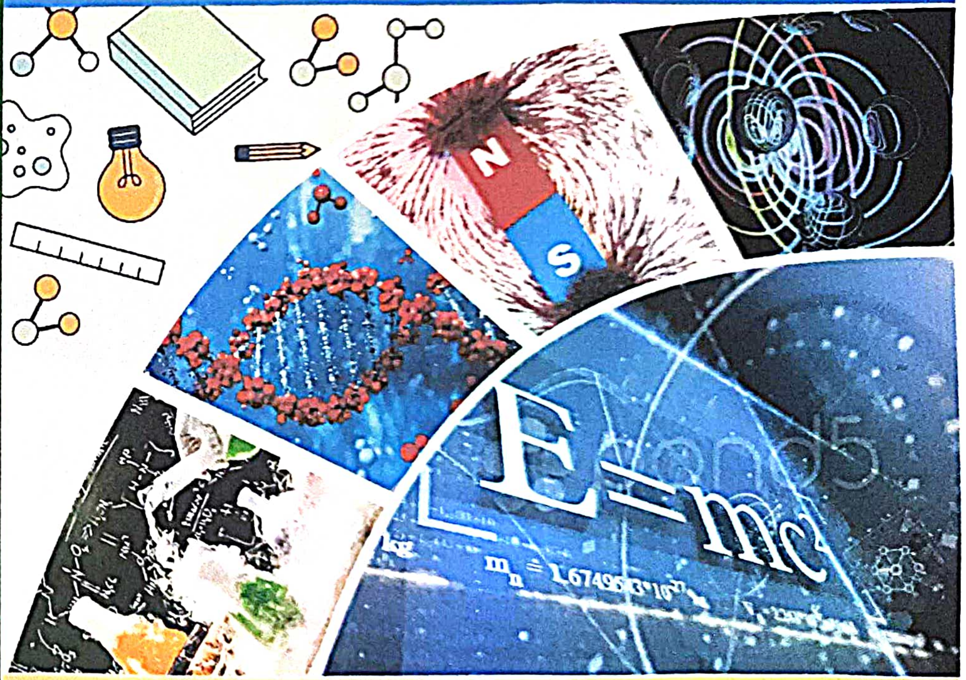

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