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A review on biogenic synthesis of gold nanoparticles, characterization, and its applications

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ABSTRACT

The nano-sized particles make an imprint on us in our daily lives and it has great importance in the numerous fields of biotechnology like the food industry, medical and industrial field. Gold nanoparticles are one of the widely used particles as it has many therapeutic applications, such as drug delivery system for many diseases like cancer, cardiovascular diseases, diabetes mellitus etc. biosensors, and environmental applications of dye degradation, bioremediation of toxic chemicals present in the environment (soil and atmosphere). Gold nanoparticles synthesis by the green route has become the latest development, because of the bioavailability of sources like plants or microorganisms, and it also reduces the utilization of toxic chemicals. This review explains the various microorganisms like bacteria, algae, fungi, actinomycetes and yeast involved in the synthesis of these nanoparticles also elucidate the size, shape and functional groups involved in the synthesis of nanoparticles and its applications.

Introduction

Nanotechnology, a combination of principles involving biology, physical and chemical that creates nano-sized particles holding particular functions [1–4]. For this purpose, noble metal nanoparticles like silver, gold, platinum, palladium etc. and non-metallic, inorganic oxides like the zinc oxide, titanium oxide have been widely exploited because of their unique electronic, mechanical, optical, chemical and magnetic properties [5–10]. The nanoparticles have unique properties of exhibiting larger surface area to volume ratio, size, shape like spherical or rod, etc. due to which they are being used in the various fields of diagnostic biological probes, optoelectronics, display instruments, catalysis, fabricating biological sensors, diagnosis or monitoring diseases like cancer cells, drug discovery, detecting environmental toxic metals or reagents and in therapeutic applications [11–15]. For the synthesis of nanoparticles, there has been an increase in the development of healthy and environment-friendly methods which don't require the exploitation of the toxic chemicals. The growth of metal nanoparticles using physical or chemical methods are not gracious or healthy owing to the use of reducing agents which are highly reactive or toxic in nature for human consumption or to the environment, and these are also quite expensive for upscale production. The green synthesis involves microbes as reducing agents like fungi, bacteria, algae, virus and plants amongst which algae, is known as the "bio-nano factories" as it is environmentally effective, affordable, are uniquely structured, macroscopic and have a high capability of metal uptake [13–17]. The toxic chemicals produced during the nanoparticle synthesis can easily be degraded with the help of enzymes present in the microbes or plants. For example, in the case of fungi, the nitrate reductase is involved in the nanoparticle reduction [18,19].

The reduction of Au (III) ion to Au atom involves binding of the atom to the cell surface, while other reduced Au also binds and aggregates to form gold nanoparticles. When it comes to bulk, gold (Au) is considered to be an inert or non-reactive metal for many chemical reactions, but when gold is synthesized in nano-sized particles, they have many unique properties, like the localized surface plasmon resonance (LSPR), the electronic properties like electronic motion with spatial length scale also decreases with size, the change in individual localized levels of energy and the novel unique properties with the quantum size effects have drastic chemical changes in the transition from bulk to nano size particles [20,21]. As shown in the Fig. 1, the gold nanoparticles with different shapes produced by diverse microorganisms perform numerous functions which are associated in many fields of applications like medicine, diagnosis and therapy or cancer treatment, as anti-angiogenesis, anti -arthritic, antimalarial agents and so on. Nanocomposites of Ag- graphene, Au-graphene or Au-SnO_2 are developed on the electrochemically active biofilms (EABs), which

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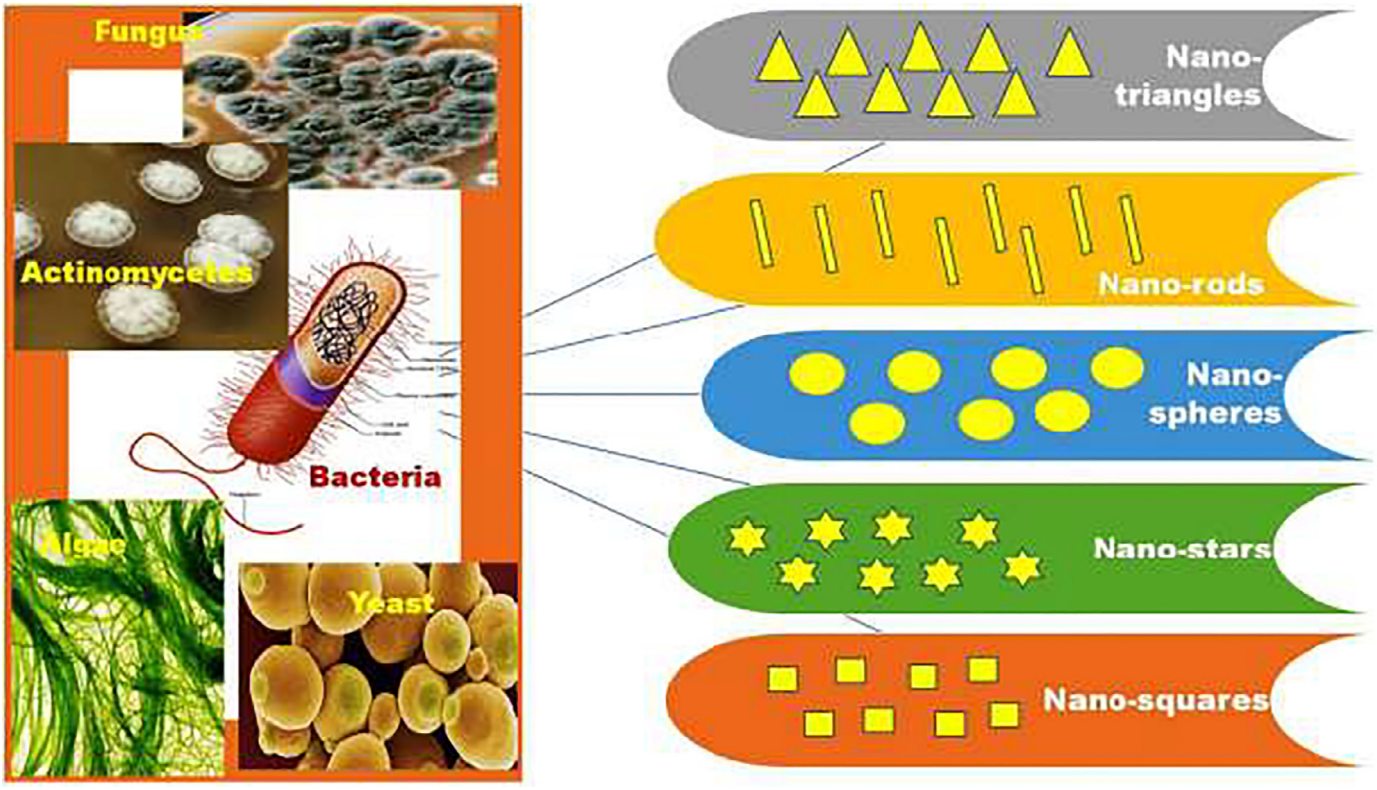

Fig. 1. The gold nanoparticle with different shapes produced from various microbial sources.

helps in the bio-reduction of the gold nanoparticles and doesn't require the use capping or surfactants for the reduction. These nanocomposites are used in various applications of sensors, photoelectrodes, optoelectronic devices, photocatalysis, photovoltaic, ultracapacitors and also photovoltaic because of their excellent photoelectrochemical and photocatalytic properties that it possesses [22–25].

Microbial synthesis of nanoparticles

Microbes have been used for the synthesis of nanoparticles due to their ease of handling, growing in a low-cost medium like cellulosic wastes or wastelands, maintaining the safety levels, having the potential of adsorbing the metal ions and reducing them into nanoparticles by the enzymes produced by metabolic processes [26,27].

The nanoparticle synthesis by microbes can be intracellular or extracellular depending upon the location. The intracellular mechanism is the transportation of specific ions into the cell wall, which is negatively charged, and with the positive charged metals they get diffused through cell wall by electrostatic attraction. Then, the enzymes present in the cell walls of the microbes convert the toxic metals into no- toxic metal nanoparticles. While, the extracellular mechanism involves enzyme mediated synthesis like nitrate reductase or hydroquinone synthesized by many fungi or prokaryotic organisms, converting the metallic ions to metallic nanoparticles. A similar mechanism was found out for gold nanoparticles synthesized from *Rhodomonas capsulate* [28,29]. The detoxification mechanisms employed by the microorganisms includes vacuole compartmentalization, metal binding or volatilization i.e. converting metals into volatile states.

When the microbes are under metal-stress situations, for survival they perform various mechanism to eliminate the heavy toxic metals. It involves an active efflux of metallic ions through the cell membranes, reduction of toxic metals ions to non- toxic ions, and also accumulating the metal ions within the cells. The heavy metal like gold, silver, lead, nickel and etc. influx is mediated through ion pumps, carrier mediated transport, endocytosis, ion channels or lipid permeation [30]. Chelating agents like siderophores are small ion binding molecules that chelate heavy metals, mediate absorption and helps in transportation from the cell of the microbes [31]. Molecules like glutathione which are derived peptides (phytochelatin) binding metals [32] or Metallothioneines (MTs), a cystien – rich protein, low molecular weight which are isolated from *Syneococcus sp., Pseudomonas putida, Cyanobacterium* and *E. coli*, perform primary function of metal detoxification [33].

Synthesis by bacterial strains

The Fe(III)-reducing bacteria like *Geobacter sp. Magnetospirillum magnetotacticum,* and so on can be used for bio-remediation of toxic metals like Fe (III) through reduction, where iron is actively taken by the cell, re-oxidized to hydrous oxide (low density) to Fe(III) oxide (ferrihydrite), which is of high density. The Fe(III) ions in the last step is reduced and magnetite is produced from dehydration within the magnetosome vesicles. An intracellular protein Ferritin, accumulates the iron within the vesicles keeping it in non-toxic and soluble form. The nanoparticles produced have following characteristics like high purity, little crystalline defects, narrow size, mono-dispersive and so on [29]. The thermophilic bacteria can be an excellent tool for the extracellular synthesis of both gold and silver nanoparticles. These extracellular systems produce an environment-friendly alternative for huge quantities of nanomaterials reducing the downstream processing of these metals [33]. The MDR (multi-drug resistance) bacteria have developed antibacterial agents that act against gram positive or negative bacteria. A known fact, that gram negative has a very thin peptidoglycan layer of cell wall which is susceptible to the action of nanoparticles

when compared to the gram-positive bacteria that possess a thick layer of the cell wall that shows stronger resistance against antibacterial agents. So, therefore with the help of the gold nanoparticles there is a possibility of also acting against the gram-positive bacteria [34].

Synthesis by fungal strains

In this recent day, a wider variety of fungi have been exploited as they are found to be a potential player in the biogenesis of the nanoparticles like the gold. They are widely used as they secrete larger bulks of enzymes that have many advantageous applications and can be worked in the laboratory [35]. The filamentous fungi have unique advantages over other microorganisms like bacteria and algae, as they have high metal tolerance and have the capability of bioaccumulation. They are helpful in the scale-up, handling of biomass, downstream processing, economic viability and they also secrete extracellular enzymes, of which large scale production is easily possible. The biochemical composition, shape and size distribution of the nanoparticles are controlled by the active biomolecules produced by the fungal organisms. The gold ions were absorbed by them and that led to the formation of the gold nanoparticles produced intracellularly. The active molecules involved can be reducing sugars, proteins, like ATPase, glyceraldehyde-3-phosphate dehydrogenase, 3-glucan binding proteins; all are involved in the energy metabolism of the cells of the fungi. The Au-fungal cells ultrathin sections when studied, it was found that gold nanoparticles were gathered in the vacuoles of the cells [36].

Synthesis by actinomycete strains

The actinomycetes have been differentiated as prokaryotes and can be easily modified genetically for the achievement of better size and poly-dispersed nanoparticles [37]. The actinomycetes have a closer resemblance with the fungi and the prokaryotes characteristics like the bacteria (mycobacteria and the coryneform). They are currently being used in the nanotechnology as they have the ability to produce secondary metabolites like antibiotics [38].

Synthesis by algal strains

Algae, the photoautotrophic, eukaryotic, aquatic, oxygenic microorganism has the ability to accumulate heavy metals, due to this fact; researchers are finding cleaner techniques for the preparation of nanoparticles. This represents a good advantage of using algae as an abundant raw material source [39]. Fucoidans are polysaccharide secreted from the cell walls of marine brown algae and that has proved to possess many applications in diverse fields like the anti-coagulant, anti-inflammatory, anti-viral and also anti-cancer. They are also being used in the cosmetic industries as an anti-aging or whitening agents. The synthesis of gold nanoparticles from these fucoidans has proved to a fruitful alternative to the chemical methods [40]. The brown algae has been exploited more as compared to other species due to its ability of uptake of heavy metals. They have a complex cell wall which is rich in mucilaginous polysaccharides, which explain the heavy metal uptake clearly. Also, it contains functional groups like the carboxyl groups, which are involved in the uptake [41].

Synthesis by yeast strains

The yeast *Hansenula anomala* has the ability to donate electrons and can act as a catalyst in the case of biofuel. The reductants are extracted from the yeast and used as a reagent for the preparation of gold nanoparticles [42,61]. However, very few yeast strains have been used in the investigation of gold nanoparticles [43].

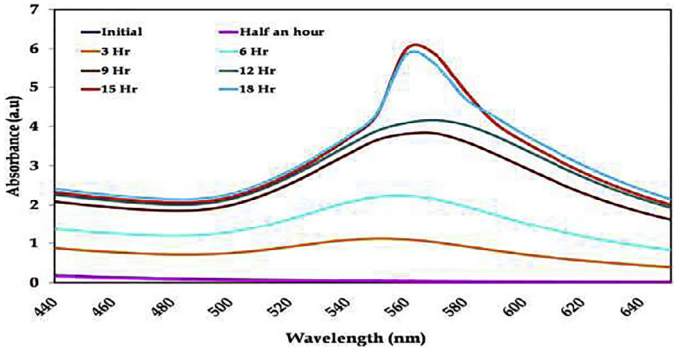

Fig. 2 . The absorption spectra of gold nanoparticles using marine *Entrococcus sp.* [46].

Characterization of nanoparticles

Visual color and UV-visible analysis

The characterization of these gold nanoparticles begins with a visual color change which works on the principle of surface Plasmon resonance (SPR). The color change occurs when the size of the particles increases, in the case of gold it is from deep red to purple. The varying color changes are due to LSPR that they exhibit, and it lies in the visible region of the electromagnetic spectrum, which means that particular portion of the wavelength in the visible region is absorbed while another portion gets reflected and the emitted wavelength will reflect its own color. The absorbance of these color changes is measured using UV-Visible spectroscopy [43]. The characteristic optical property exhibited by the metallic nanoparticles is due to the oscillations of the conduction band of electrons surfacing the nanoparticles. For example, when the bacterium *E.coli* was resuspended in distilled water, it demonstrated a milky white color prior to the addition of the diluted solution of $HAuCl_4$, with the addition it shows pale yellow color which is the color of HAuCl4 solution. But, after the bacterium is incubated, the solution turns colorless indicating that the bacterium has assisted in the reduction of gold nanoparticles [67]. Thus, proving the color changes [44]. The gold nanoparticles synthesized using *Klebsiella pneumoniae* produced SPR values in the range of 400–700 nm [45]. As shown in Fig. 2 the absorption spectra of 545 nm was observed for gold nanoparticles from marine *Entrococcus sp.* The peaks also demonstrate the stability of the nanoparticles which increases with time [46].

SEM analysis

The analysis from Scanning Electron Microscope (SEM) requires sample preparation, which includes the formation of thin films of carbon coating on copper grids. These films were prepared by dropping a minute amount of sample onto the grip while the remaining solution was removed with blotting paper, followed by further drying under the mercury lamp for a minimum of 5 min only [47]. As shown, in the Fig. 3 the SEM images demonstrating different shapes of the gold nanoparticles using the *Turbinaria conoides.* The gold nanoparticles were found to be in various shapes like the rectangle, square, cubic and also triangular and the average diameter was 60 nm [48].

TEM analysis

The sample preparation for TEM characterization involves placing a drop of solution on a carbon- coated copper grip which was dried at a room temperature, while the residual solution was removed with the blotting paper. The TEM gives information on the

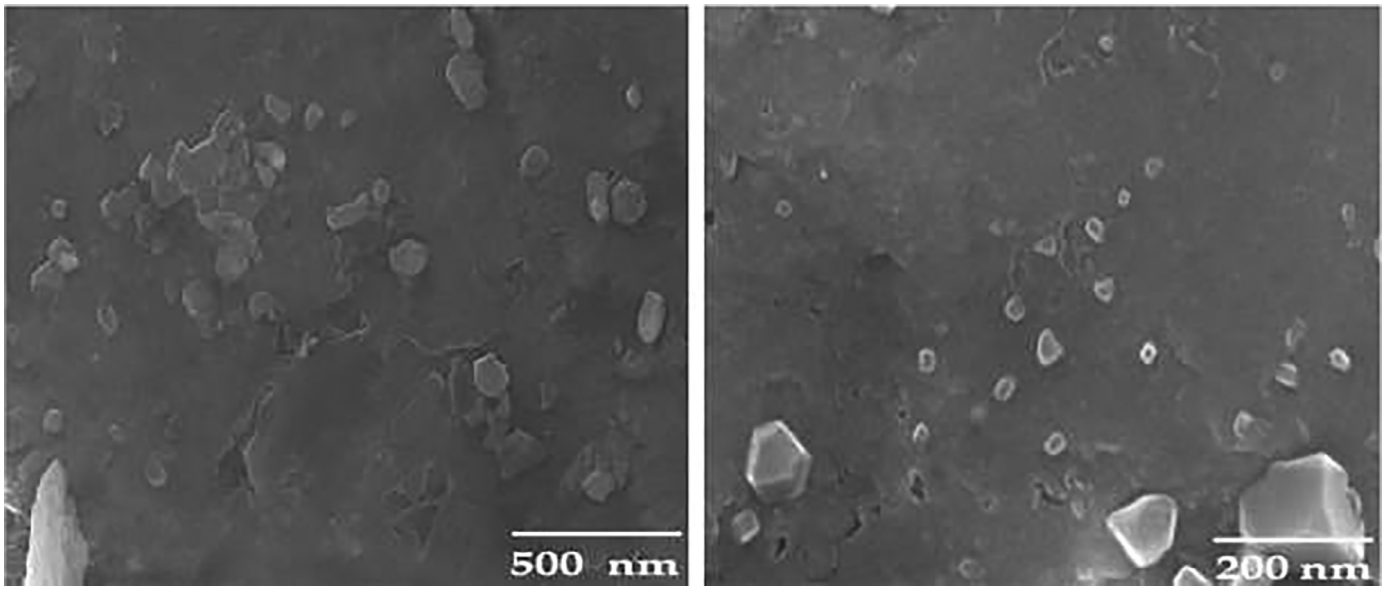

Fig. 3. SEM images of functionalized gold nanoparticles synthesized by using *Turbinaria conoides* [48].

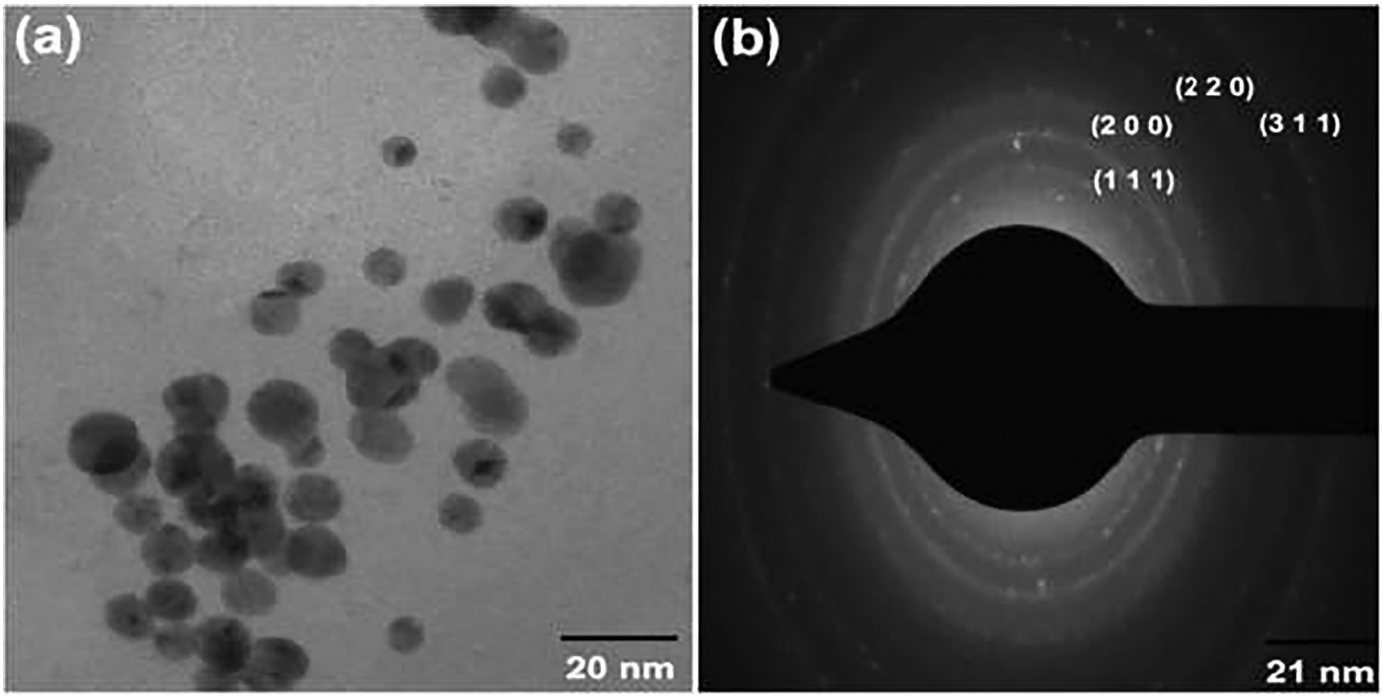

Fig. 4. HR-TEM images of gold nanoparticles formed by bacteria (a) 50 nm scale and (b) selected area diffraction pattern [46].

morphology and shapes of these nanoparticles [49]. As shown, in the Fig. 4 the TEM image of marine *Entrococcus sp.* on the right depicts the uniformity of the spherically shaped nanoparticles with average size of 10 nm. While the SAED pattern on the left confirms the crystalline nature of the nanoparticles [46].

EDX analysis

EDX (energy-dispersive X-ray spectroscopy) is a technique used for the chemical characterization or elemental analysis of any given sample. It can be used to basically determine the number of gold nanoparticles produced with a thin film of bacterial biomass [50].

AFM analysis

The AFM imaging was done using the phosphorus-doped silicon probes, while the sample was equipped by dissolving the bio-reduced nanoparticles in either ethanol or water, and then a droplet of the solution was added on the pre-cleaned substrate of silicon. It was allowed to dry gradually. The Si- substrate comprising the sample was used for AFM imaging and further measurements [51].

FTIR analysis

FTIR measurements were reinforced to identify the probable biomolecules which can be liable for capping leading to proficient

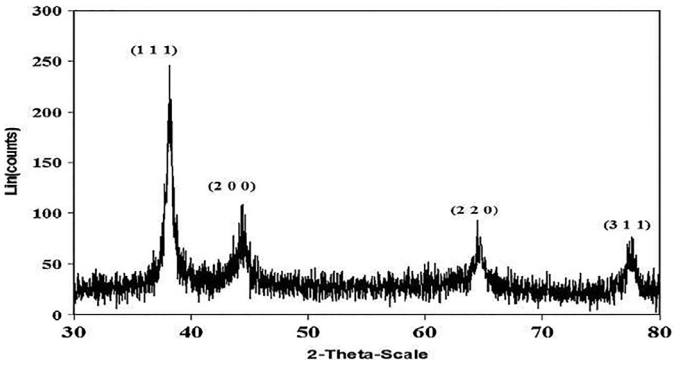

Fig. 5. XRD diffraction pattern of gold nanoparticles using *Klebsiella pneumoniae* [48].

stabilization of the gold nanoparticles [52]. The refined suspension containing the nanoparticles was completely dried and ground with KBr pellets and studied. In order, to obtain decent signal/noise ratio, 512 scans are verified to get appropriate results [53].

XRD analysis

The crystalline nature of gold nanoparticles was established using X-ray diffraction (XRD) analysis [54]. The sample preparation involved the reduced the gold nanoparticles solution to be drop-coated on a glass surface and was done on the equipment which was effective at the voltage of 40 kv, and running at the voltage of 20 mA with Cu Kα radiations [55]. In the Fig. 5, the crystalline nature of the gold nanoparticles using *Klebsiella pneumoniae* has been analyzed with the XRD diffraction pattern and the mean size of these nanoparticles was calculated using Debye-Scherer equation [48].

Applications

As, cancer is the disease which has been affecting almost every human life now, researchers have been working on gold and silver nanoparticles for its treatment. The nano-sized anticancer agents have been standardized against a variety of human cancer cells like prostate, colon, lung, cardio and breast cancer [56]. Gold nanoparticles have also found its applications in the manufacturing of biosensors due to their electron transferring ability and an adsorptive capacity. Nanoparticles have been used for sensing different types of toxic metals from the environment. For example, copper is considered as a toxic pollutant, also involved in the genetic disorder Wilson's disorder in which the copper level is above than normal, which is required for physiological activities, the gold nanoparticles have been used in the detection of such pollutants [57,71].

The antimicrobial activity where the interaction between the nanoparticles and microorganism is exploited, due to the surface -volume ratio, size or shape of the nanoparticles, it changes the permeability of the cell membrane of the bacteria or the fungi by creating gaps or pits, thus inhibiting the enzymatic activity of the respiration leading to apoptosis of the cells [58].

Recently, the gold nanoparticles are found to be a great help in catalysis of the production of hydrogen mediated through microbial fuel cells (MFCS) when placed at the cathodes, by using the quantized charging effect and not with the use of any external power supply [59]. The nanocomposite structures have a comparatively larger surface area, better conductivity, structure and etc. with these applications it is used as photocatalyst and for photocapacitive studies [60] (Table 1).

The blue- green bacterium *Spirulina platensis*, the particle size was determined using HR-TEM (high-resolution transmission electron microscopy) with an average size of 5 nm with a spherical shape. While, thermophilic bacterium *G. Stearothermophilus* has revealed that the size of nanoparticles was 11 nm, 12–14 nm and also few visualized 5–8 nm of particle size which was determined using TEM. The *P. denitrificans* belongs to Pseudomonadaceae family, it synthesized nanoparticles of size 25–30 nm which was analyzed from both HR-TEM and the SPR (surface plasmonan resonance) peaks of UV-Visible spectra showed the spherical shape at optimum pH 3 at room temperature, while the sulphydryl, amide, carbonyl groups were found to be involved in increasing the stability of the particles of the FTIR results. The bacterial strains of the Enterobacteriaceae family, synthesized nanoparticles of spherically shaped mostly, other species like Bacillaceae produced triangles, and Pseudomonadaceae family produced spherical and blunt triangles. The *S. koreensis* which belonged to Planococcaceae family synthesized spherical shaped particles using the FE-TEM (field emission transmission electron microscopy), while the TEM results showed particle size of 30–50 nm and DLS analysis showed size of 92.4 nm. The change in the size of DLS and TEM is due to the fact that DLS can measure the hydrodynamic diameter, but TEM can visualize the metallic core of that nanoparticle. The *Shewanellao neidensis* produced gold nanoparticles of size 2–50 nm with the help of TEM. The probable functional groups involved in the synthesis are N–H groups, carbonyl stretches and etc. indicating the proteins, peptides might be involved in the stabilization or reduction of the nanoparticles (Table 2).

The data collected for *Aspergillus foetidus* from UV-vis absorption is used only for the purpose of finding the maximum absorption, indicating that the nanoparticle intensity is highest in that range and the SPR (surface Plasmon resonance) analysis reveals the color change into deep pink, which shows a high concentration of nanoparticles. The size and shape were confirmed, by using first 20–50 nm of size using AFM analysis, 30–50 nm of size from FE-SEM (field emission scanning electron microscopy) and final confirmation of size 10–40 nm from the TEM analysis.

The gold nanoparticles prepared from *Trichoderma sp* were spherical in shape and have good antimicrobial potential [97].

The experiment using the fungal strain *Penicillium oxalicum* performed for the synthesis of gold nanoparticles had revealed that average size particle detected from TEM and DLS analysis at pH of 8 and 12 was nearly 6 and 4 nm. It was noticed that TEM results gave smaller particle size than DLS analysis, which might be due to the aggregation of the nanoparticles. They found that optimum pH of 8 and 12 gave the best results, and confirmed the size and its distribution of particles using the DLS analysis. But, no FTIR analysis was performed to know the functional groups involved.

The fungal strain *Pycnoporus sanguineus* synthesized nanoparticles of different size but in the range of few 100 nm [92]. With the change of gold concentration from 0.5 mM to 2.0 mM, the size varied from 25.88 to 51.99 nm, and with the change of pH also gave different sizes, i.e. at pH 2 the size was 84.29 nm and at pH 12 the size was 6.07 nm. The TEM also gave results of various shapes like spherical, pseudo-spherical, triangular, and truncated triangular (Table 3).

The TEM analysis had revealed that algal (*Galaxaura elongata*) mediated nanoparticle synthesis produced various shapes which included spherical (dominantly), rod, triangular or truncated triangular and even hexagonal (few). While, the FTIR results confirmed the presence of carbonyl stretch and N–H stretch having a stronger potential to bind with the metal nanoparticle, helping in the formation of a coat, that prevents the particle from agglomeration. The algal extract andrographolide, alloaromadendrene oxide, glutamic acid, hexadecanoic acid, oleic acid, eicosenoic acid, stearic acid, gallic acid, epigallocatechin catechin and epicatechin gallate

Table 1
Bacterial mediated synthesis of gold nanoparticles.

S. No	Bacterial strain	Family	Size (nm)	Shape	Functional groups	Reference
1.	*P. aeruginosa* (ATCC 90,271) *P. aeruginosa* (2) *P. aeruginosa* (1)	Pseudomonadaceae	50–30 (TEM)	–	–	[53]
2.	*Pseudomonas denitrificans*	Pseudomonadaceae	25–30 (HRTEM)	At 37 °C pH 3 roughly spherical (blunt shaped nano-triangles)	Sulphydryl, amido, carbonyl	[54]
3.	*Pseudomonas fluo-rescens* 417	Pseudomonadaceae	5–50	Spherical	Hydroxyl, carbonyl, aromatic groups	[55]
4.	*Pseudomonas veronii* AS41G	Pseudomonadaceae	5–25 (TEM)	–	NH2 symmetric CO stretching primary amide C=O symmetric C–Cl stretching	[56]
5.	*Rhodopseudomonas capsulata*	Rhodospirillaceae	10–20 (TEM at pH7)	Spherical (at pH 7)	–	[57]
6.	*Shewanella algae* (ATCC 51181)	Shewanellaceae	9.6 after 1 h(mean size), 100 nm after 6 h, 100–200 nm after 24 h (TEM)	Spherical	Carbonyl group (C=O)	[66]
8.	*bacillus*	Bacillaceae	20–50 (SEM) at pH7	Spherical	Amino, sulfhydral and carboxyl groups	[43]
9.	*Escherichia coli* *Citrobacter freundii* *Citrobacter koseri Proteus vulgaris* *Serratiamarcescens* *Enterobacter sp.* *Klebsiella pneumoniae* *Proteus mirabilis* *Klebsiella oxytoca*	*Enterobacteriaceae*	11.8–130 321 32–127 25–369 18.3 89 459 24–256 110 20–400 (SEM)	Spherical (SEM)	amide linkages and –COO–	[15]
10.	*Escherichia coli* DH5a	Enterobacteriaceae	20 (XRD, TEM)	Spherical	–	[36]
11.	*Escherichia coli*	Enterobacteriaceae	5–20 (TEM, SEM)	Spherical	SH (thiol group)	[65]
12.	*Klebsiella pneumonia* *Lactobacillus amylotrophicious* *Salmonella enterica*	Enterobacteriaceae Lactobacillaceae Enterobacteriaceae	5–65 (TEM)	Spherical	Amine groups or carboxylate groups	[3]
13.	*Bacillus stearothermophilus*	Bacillaceae	5–30 (TEM)	Triangle and other shapes	–	[27]
14.	*Spirulina platensis*	Phormidiaceae	~5 (HR-TEM)	Spherical	–NH functional group	[30]
15.	*Stenotrophomonas maltophilia*	Xanthomonadaceae	~40	Spherical	_	[70]
16.	*Geobacillusstearo thermophilus*	Bacillaceae	11,5–8, 12–14 (TEM)	Spherical	Amide I and II (due to CO stretch and –N–H stretch vibrations in the amide linkages of proteins)	[33]
17.	*Magnetospirillum Gryphiswaldense* MSR-1	Rhodospirillaceae	10–40 (TEM)	Spherical	–	[14]
18.	*Shewanellao neidensis*	Shewanellaceae	2–50 (TEM)	Spherical	–N–H stretch and carbonyl (–C–O–C– or –C–O–) stretch vibrations in amide linkages (amide I and amide II) amide III was also observed (implying the presence of protein/peptide) carbonyl and hydroxyl functional groups in alcohols and phenol derivatives	[2]
19.	*Sporosarcina koreensis* DC4	Planococcaceae	30–50 (TEM) 92.4(DLS)	Spherical (FE-TEM)	–	[69]
20.	*Staphylococcus epidermidis*	Staphylococcaceae	20–25 (TEM)	Spherical	Amine or carboxyl	[68]

Table 2
Fungal mediated synthesis of gold nanoparticles.

S. No	Fungal strain	Family	Size (nm)	Shape	Functional groups	Reference
1.	*Aureobasidium pullulans, Fusarium oxysporum* and *Fusarium*	Dothioraceae Nectriaceae	35–23	Spherical	Amide II and aldehydes (*A.pullulans*)	[36]
			208–43		C =O stretching frequency and C=C double bonds (*Fusarium* and *F.oxysporum*)	
			198–58			
2.	*Alternaria alternata*	Pleosporaceae	2–30	Spherical and triangular	O–H stretching, C–H stretching (proteins and other organic residues), amide I (polypeptides) amide III bands (the random coil of protein)	[13]
3.	*Botrytis cinerea*	Sclerotiniaceae	1–100	Triangular, hexagonal, spherical, decahedral,and pyramidal	–	[82]
4.	*Penicillium crustosum*	Trichocomaceae	100 (AFM)	Spherical	Amide, carboxylic stretch (methylene groups of the protein) N–H bend (primary amines, carbonyl stretch in proteins)	[83]
5.	*Penicillium Chrysogenum*	Trichocomaceae	5–100	Spherical, triangle and rod	–	[87]
6.	*Penicillium oxalicum*	Trichocomaceae	4 (at pH 12.0) 6 nm (at pH 8.0) (TEM)	Spherical	–	[88]
7.	*Phanerochaete Chrysosporium*	Phanerochaetaceae	10–100	Spherical	Amino and sulfhydryl	[89]
8.	*Pycnoporus sanguineus*	Polyporaceae	25.88–51.99 with initial change of gold concentration from 0.5 to 2.0 mM	Spherical, pseudo-spherical, triangular, truncated triangular, pentagonal, and hexagonal (TEM)	Hydroxyl, amine and carboxyl	[90]
			84.29 at pH 2, 6.07 at pH of 12 (TEM)			
9.	*Rhizopus oryzae*	Mucoraceae	5–65	Spherical	Carboxyl and amine	[91]
10.	*Neurospora crassa*	Sordariaceae	3–100	Spherical	–	[84]
11.	*Fusarium semitectum*	Nectriaceae	10–35	Spherical	Amide I and amide II	[85]
12.	*Fusarium solani*	Nectriaceae	20–50 (TEM)	Spherical	C–O stretching	[86]
13.	*Aspergillus foetidus*	Trichocomaceae	30–50 (FESEM 10–40 (TEM)	Spherical	Amine groups or carboxyl groups of the cysteine residues in the protein	[81]
			20–50 (AFM)			
14.	*Trichoderma harzianum*	Hypocreaceae	26–34 (TEM)	Spherical	N–H stretching, –OH group from the carbohydrates or protein, –SH group indicating the presence of cysteine in the biomass.	[94]
15.	*Phanerochaete Chrysosporium*	Phanerochaetaceae	10–100 (AFM)	Spherical	–SH-stretching	[89]
16.	*Sclerotium rolfsii*	Atheliaceae	25 (TEM)	Spherical	Amide I, the carboxyl group, C–N stretching from amines.	[93]
17.	*Trichodermaviride hypocrealixii*	Hypocreaceae	20–30 and 120 at 30 °C At 100 °C below 20.	Spherical	–	[95]

Table 3
Algal synthesis of gold nanoparticles.

S.No	Algal strain	Family	Size (nm)	Shape	Functional groups	Reference
1.	*Cladosiphonokamuranus Kjellamaniella crassifolia*	Chordariaceae	8–10	Spherical	Sulfate	[38]
2.	*Pithophoraoedogonia*	Pithophoraceae	32.06	Spherical	–	[72]
3.	*Padina gymnospora*	Dictyotaceae	53–67	Spherical	Hydroxyl	[73]
4.	*Padina tetrastromatica*	Dictyotaceae	8.3–25	Thin planner	Carbonyl	[74]
5.	*Stoechospermum marginatum*	Dictyotaceae	18.7–93.7	Spherical in shape and some are hexagonal and triangle	Hydroxyl groups	[75]
6.	*Chondrus crispus*	Algaceae	30–50 (increase of initial pH)	Polyhedral and spherical (at pH 4) polygonal (triangular and hexagonal) (at acidic medium) nanospheres(basic condition)	Amide I and II bands, methylene scissoring vibrations of proteins, COC antisymmetric stretching, COC symmetric stretching and C = S stretching	[37]
7.	*Sargassum wightii*	Sargassaceae	8–12	Thin plannar	–	[79]
8.	*Sargassum swartzii*	Sargassaceae	14–70 (DLS)	Spherical (HR-TEM micrograph)	Carboxylic group (C–O) stretch	[78]
9.	*Ecklonia cava*	Lessoniaceae	30 ± 0.25	Spherical and triangular	Primary amine The group, hydroxyl group	[39]
10.	*Spirulina platensis*	Phormidiaceae	20–30	Spherical	–	[77]
11.	*Turbinaria conoides*	Sargassaceae	–	–	Hydroxyl groups	[80]
12.	*Galaxaura elongata*	Chaetangiaceae	3.85–77.13 (Zeta potential)	Spherical in shape along with a few rods, triangular, truncated triangular and hexagonal shaped (TEM)	Carbonyl stretch and free N–H stretch Vibrations in the amide linkages of the proteins.	[6]
13.	*Prasiola crispa*	Prasiolaceae	5–25 (TEM)	Spherical	N–H, C–N stretching vibrations, C–H stretching and carboxylate groups.	[76]

identified may help in reducing, then stabilizing and capping or covering agent. And the surface charge and stability of the particles was confirmed by the zeta potential.

The specimen taken was a freshwater epilithic green alga, *Prasiola crisp*, with a size of 5–25 nm and spherical shaped both measured from TEM analysis. The FTIR analysis showed N–H and C–N is stretching vibrations of primary aliphatic amines, C–H stretching corresponding to amide and carboxylate groups found in amino acid residues. Hence, the presence of organic molecules and functional groups of the protein molecules proved to provide stability and preventing nanoparticles from agglomeration.

The *Sargassum swartzii* algal strain produced gold nanoparticles of size 14–70 nm measured from DLS proved that the particle size is comparatively larger when compared to the TEM analysis, which was found to be in the range 20–60 nm measured from HR-TEM. The FTIR results had confirmed the presence of carbonyl group involved in the reduction of nanoparticles

Stoechospermum marginatum, a brown alga, produced gold nanoparticles measured from TEM and the FTIR analysis showed that the reduction is possible due to the terpenoids containing the hydroxyl group present the seaweed (Table 4).

The gold nanoparticles synthesized from *Gordoniaamarae* was analyzed using UV -visible spectroscopy, XRD, TEM, Energy Dispersive Spectra (EDS) and Selected Area Energy Dispersion (SAED) patterns revealed that they are produced nanoparticles optimally at a pH of 10 at 90 °C for a maximum of 20 min, the shape was found to be spherical. The gold particles produced from *Streptomyces sp.* VITDDK3 was found to have maximum 90 nm size having a cubical shape when compared with other *Streptomyces* species. The *Thermomonospora sp.* has the minimum size of 8 nm of the nanoparticle. The data collected from M.E. Castro et al experiment revealed that extracellular NADH-dependent reductase was involved in the reduction of gold nanoparticles, but no FTIR analysis was performed to prove this data. And, the TEM analysis revealed the size and shape of gold nanoparticles produced by *Botrytis cinerea* (Table 5).

Little work has been reported for the yeast mediated gold nanoparticle synthesized, among which the synthesis using *Candida guilliermondii* produced spherical shape, of size 50–70 nm, while *Yarrowialipolytica* which belongs to Dipodascaceae family produces nanoparticles of size 15 nm with hexagonal, triangular shape nanoparticles. And also, *Magnusiomycesingens* LH-F1 which also belongs to the same Dipodascaceae family produced nanoparticles of different size of 9.8–80.1 nm having shapes of spheres, triangle and hexagon. The functional groups of amide and carboxyl were said to be involved in the reduction of the nanoparticles.

The main drawback in the chemical method employed for the nanoparticle synthesis, is the use of toxic chemicals or reagents [103–115]. The (Table 6) depicts the various conventional methods for gold nanoparticle synthesis using chemical or physical studies. It is seed that the size and shape of the nanoparticles are well maintained, i.e. less than 100 nm, but the temperature used in the reaction is quite high nearly 100 °C, and the chemicals used in the chemical methods or the equipment used in the physical means like laser or sonication treatment are expensive, and also toxic and not environment friendly. The physical methods require man power when compared with chemical or biological methods. And, for green methods it discourages the use costly chemicals, also consuming less energy, produces environmentally products and byproducts. Since, there is a huge plethora of flora and fauna available in the environment, there is also no limitation with the resources.

Table 4
Actinomycete synthesis of gold nanoparticles.

S. No	Actinomycete strain	Family	Size (nm)	Shape	Functional groups	Reference
1.	*Thermomonospora*	Thermomonosporaceae	8	Spherical	Amide I and II bands of proteins	[35]
3.	*Streptomyces hygroscopicus*	Streptomycetaceae	20	Spherical (TEM)	–	[55]
4.	*Gordoniaamarae*	Gordoniaceae	15–40 (pH10.0, 90 °C for 20 min)	Spherical	–	[49]
5.	*Gordoniaamicalis*HS-11	Gordoniaceae	5–25	–	O–H stretching, C–H stretching of alkanes, C=O stretching, O–H bending for carboxylic acid.	[58]
6.	*Streptomyces fulvissimus*	Streptomycetaceae	20–50	Spherical	–	[44]
7.	*Streptomyces sp.* VITDDK3	Streptomycetaceae	90 (SEM)	Hexagonal, cubical, brick and irregular (SEM)	–	[63]
8	*Thermomonospora*	Thermomonosporaceae	8	Spherical	Amide I and II bands of proteins	[35]
9	*Streptomyces viridogens(*HM10)	Acidothermaceae	18–20	Spherical and rod (TEM and XRD)	–	[42]
10	*Streptomyces hygroscopicus*	Streptomycetaceae	20	Spherical (TEM)	–	[62]
11	*Gordoniaamarae*	Gordoniaceae	15–40 (pH 10.0, 90 °C for 20 min)	Spherical	–	[52]

Table 5
Yeast- mediated synthesis of gold nanoparticles.

S. No	Yeast strain	Family	Size (nm)	Shape	Functional groups	Reference
1.	*Candida guilliermondii*	Saccharomycetaceae	50–70	Spherical	–	[64]
2.	*Yarrowialipolytica* (NCIM 3589)	Dipodascaceae	15 nm (at pH 7.0 and 9.0)	Hexagonal and triangular	–	[96]
3.	*Magnusiomycesingens* LH-F1	Dipodascaceae	80.1 ± 9.8 (TEM) 137.8 ± 4.6 (DLS)	Sphere, triangle, and hexagon	Amide and carboxyl groups	[41]

Table 6
Chemical and physical mediated synthesis of gold nanoparticles.

S. No	Precursor or method	Size (nm)	Shape	Temperature °C	Reference
1.	Citrate reduction (Chemical)	15–20	Round or spherical	97	[98]
2.	1-amino-2-naphthol-4-sulphonic acid (ANSA) (Chemical)	35.1	Hexagonal, pentagonal, spherical, etc.	RT	[99]
3.	Poly(styrene-block-4vinylpyridine)(PS-P4VP) and 2(-4-hydroxyphenylazo)benzoic acid (HABA) block copolymer (Chemical)	12 (diameter) (AFM)	Hexagonal	–	[100]
4.	Femtosecond laser irradiation method (physical)	Larger than 20 When irradiation was increased to 10min	Spherical	RT	[101]
5.	Ultrasonication-hydrothermal reaction (US-HT) (physical)	5–10	Octahedral	–	[102]

Conclusion

There are abundant microbes present in the environment, so the screening of every potential microbeis very important, the compounds involved in the mechanism of nanoparticle synthesis from these microbes should also be considered. Therefore, the physiochemical and purification characterization of these bimolecular compounds can be analyzed. The aggregation of nanoparticles is also a very important factor which should be strictly checked because the aggregated particles may give varying results. So hence, stabilization of these nanoparticles by novel stabilizing agents can be implemented in the synthesis. The emerging field of bio-nanotechnology has unlocked many different paths for the progress of novel products which can be helpful for human beings. Hence, further research has been going on to explain the mechanism for the synthesis of these particles, and how it be can be used in the treatment of rare diseases, also including all the types of cancer. The use of polymer nanocomposites which comprises of metal nanoparticles and nano- clay can be helpful in the packaging process or preserving vegetables, fruits, beverages and other food items. With the addition of various nanoparticles to such nanocomposites it helps in building up of strong mechanical, with high thermal performance and barrier. Thus, preventing the entry of bacteria and pathogenic microorganisms and also diminishing the usage of non-biodegradable plastics. They can also act as nano-sensors that can help in the detection of spoilage of food or toxic metals present. These are the few future aspects for the generation of nanoparticles using green synthesis and yet many more is yet to be explored by the researchers.

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