Nanomaterials for Sustainability: A Review on Green Synthesis of Nanoparticles Using Microorganisms

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Abstract

Nanotechnology has permeated all areas of sciences as one of the most propitious technology with the deployment of nanoparticles in environmental remediation and biomedical fields; their synthesis under greener conditions has been bourgeoned using microorganisms, plants, etc. to decrease the use of toxic chemicals. Synthesis of nanoparticles by exploiting microorganisms has opened up a new prospect at the interface of nanotechnology, chemistry, and biology enabling access via a biocompatible, safe, sustainable, eco-friendly, and reliable route; microorganisms offer crystal growth, stabilization, and prevention of aggregation thus performing a dual role of reducing and capping agent because of the presence of biomolecules such as enzymes, peptides, poly (amino acids), polyhydroxyalkanoate (PHA), and polysaccharides. Herein, the microorganisms-based synthesis of various nanoparticles comprising gold, silver, platinum, palladium, copper, titanium dioxide, zinc oxide, iron oxide, and selenium along with their appliances in waste treatment, biomedicine namely cancer treatment, antibacterial, antimicrobial, antifungal, and antioxidants, are deliberated.

1. Introduction

“Being green and clean is not just an aspiration but an action” is a small line delivered by “Christine Pelosi” (Jiwani et al. 2018) but has a bigger meaning which inspires everyone to be active in this domain (Varma 2016; Chen et al. 2020). Green and sustainable chemistry is a general strategy for the deployment of chemicals, reagents, solvents, and processes that helps in the reduction and use of hazardous chemicals to ensure the safety of the environment (Lu and Ozcan 2015; MacKellar et al. 2020; Monga et al. 2020; Ganesh et al. 2021). Greener biosynthesis pathways for nanomaterials has garnered immense interest as nanotechnology has spread its arms in all areas of sciences as one of the most propitious technology with the deployment of nanoparticles in environmental remediation and biomedical fields (Hu and Xianyu 2021). Nanotechnology combined with green technology provides a viable alternative to physical and chemical routes by utilizing safe, renewable, and non-toxic chemicals and eco-friendly means (Silva et al. 2020; Ajayi et al. 2021). This quest continues incessantly for the modification of shape and size for enhancing the properties of nanoparticles (Zare et al. 2020). Although, the synthesis of the nanoparticles has been accomplished by alternative activation methods such as sonochemical or microwave (MW)-assisted protocols (Kou and Varma 2013; Gawande et al. 2014) and photochemical reaction techniques, laser ablation (Nasrollahzadeh et al. 2020b), lithography, etc. they often entail the use of hazardous chemicals or specialized equipment (Sarfraz and Khan 2021), various methods for the synthesis of nanoparticles are presented in Figure 1 with immense potential appliances in environmental remediation and biomedical arena (Iravani and Varma 2020; Nasrollahzadeh et al. 2021; Rabiee et al. 2021). Green nanotechnology deploying safer solvents, and eco-friendly reducing and stabilizing (capping) agents comprise biological systems wherein plants, bacteria, fungi, algae, and other microorganisms fall into this category (Venil et al. 2021b) activity during the last two decades, according to Scopus, is depicted in Figure 2 (Mohammadinejad et al. 2019).

Green synthesis using plants extracts for sustainability has demonstrated immense potential in various applications which have been reviewed earlier (Jadoun et al. 2021). On the other hand, the microbial route has also attracted significant attention due to their environmental sustainability popularly known as bio factories for nanoparticle synthesis. Additionally, the microorganisms can withstand in variables extreme conditions of pH, pressure, temperature, etc. that attracts researchers and scientists to adopt the microbial route (Vetchinkina et al. 2019). Herein, the current status of the microbial-assisted synthesis of various nanoparticles and their broader applications in environmental remediation and biomedical fields such as photocatalysis, cancer therapy, tissue engineering, drug delivery, biosensors, antimicrobial, antibacterial, antifungal, antioxidant, etc. are deliberated, Figure 3.

2. Green Synthesis Of Nanoparticles By Microorganisms

Microorganisms hold great potential for nanoparticles synthesis being cost-effective, ecofriendly, avoiding harmful toxic, and harsh chemicals along with the reduced demand for high energy usage required by physiochemical methods. Assorted reductase enzymes help in the accumulation and detoxification of metals thus reducing the metal salts to nanoparticles with minimum polydispersity and narrow size distribution (Yadav et al. 2020). Extracellular synthesis has been adopted widely and has garnered a noticeable interest due to the elimination of downstream steps of processing in the intracellular method for nanoparticles recovery as it eliminates the cell wall breakdown by sonication, and centrifugation followed by some washing stages necessary for the purification of nanoparticles. Furthermore, metal resistance genes, enzymes (Virkutyte and Varma 2011), peptides, proteins, organic materials, and reducing cofactors play a key role in green synthesis by acting as reducing agents, thus helping in the synthesis of nanoparticles by functioning as a natural capping agent and preventing the aggregation and imparting additional stability to nanoparticles for a longer period (Purohit et al. 2019; Messaoudi and Bendahou 2020; Rana et al. 2020). For nanoparticles, stabilization and surface functionalization became greener by using biocompatible stabilizing agents, i.e., microbes, which can produce vast varieties of stable flower-shaped, spherical, and rod-shaped nanoparticles that have potential uses and diverse applications. Genetically engineered organisms are the ideal choice to attain environmentally friendly, and high-throughput bio reduction for the synthesis of nanoparticles due to easy genetic manipulation and resistance to toxicity (Iravani and Varma 2019). For multidrug-resistant bacteria, metals and metal oxide nanoparticles have been extensively studied. Numerous studies suggested the antimicrobial properties of these nanoparticles against a wide spectrum of bacterial species (Niño-Martínez et al. 2019). Metal and metal oxides nanoparticles synthesized using *Lactobacillus sp.* also showed this resistance (Jha and Prasad 2010).

Numerous types of nanoparticles such as Ag (Singh 2019; Chen et al. 2021), Au (Duran and Seabra 2018; Krishnan and Chadha 2020; Botteon et al. 2021), ZnO (Sanaeimehr et al. 2018; Mohd Yusof et al. 2019), TiO_2 (Seydi et al. 2019), Cu (Lalitha et al. 2020), Se (Afzal et al. 2021; Menon et al. 2021), Pd (Arya et al. 2020), Pt (Bloch et al. 2021), and many others have been synthesized using microbes as a reducing and capping agent and these nanoparticles are applicable in wide areas.

3. Mechanism Of Green Synthesis Of Nanoparticles

In recent years, nanoparticles synthesis via microbial agents such as algae, fungi, bacteria, yeast has attracted a lot of attention for potential applications in biomedical and other fields due to their cost-effective and ecofriendly potential (Figure 4) (Gahlawat and Choudhury 2019).

The nanoparticles synthesis by microorganisms occurs either intracellularly or extracellularly. In intracellular synthesis, ion transportation takes place in the microbial cell in which the microorganism's cell wall plays a key role. The positive charge ion of metal and cell wall's negative charge is involved in the electrostatic interaction. The cell wall of microorganisms possesses the enzymes which reduce the ions to nanoparticles where the cell wall further diffuses off the nanoparticle, Figure 5 (Patil and Kim 2018). The mechanism of nanoparticle synthesis involves three steps including trapping, bio reduction, and capping (Fariq et al. 2017). Intracellular synthesis of silver nanoparticles has been performed by using *Rhodococcus sp.* which was grown aerobically in an M9 medium containing salts for 24 hours at 30°C and agitated at 130 rpm. This was used as synthesis media after 24 hours with $AgNO_3$ salt at 7.0 pH. The color changed to brown from white during this period, the incubation was performed for more than 24 hours and collected after the period. When the cell surface encounters metal ions, electrostatic interaction and trapping of ions take place. For the reduction of metal ions to metal, enzymes found in the cell wall are responsible (Otari et al. 2015).

In extracellular synthesis, firstly microorganisms are cultured in a rotating shaker for 1-2 days under optimum conditions followed by the centrifugation of culture for biomass removal, and the supernatant is collected for nanoparticles synthesis with the addition of metal salt solution (filter- sterilized) and incubated again (Ammar et al. 2021). The synthesis of nanoparticles can be visibly followed by the color change of culture medium, for example, for the gold nanoparticles (Au NPs), these color changes to deep purple color from ruby red while color changes to dark brown for silver nanoparticles (Ag NPs). The removal of large particles or medium components can be accomplished via centrifugation of the reaction mixture. Lastly, these nanoparticles can be centrifuged with a density gradient or at high speed and washed carefully with ethanol/ water, and collected (Singh et al. 2016).

4. Role Of Microorganisms

The role of microbes in the synthesis of inorganic nanomaterials with exquisite morphology is to reduce the use of harmful and toxic chemicals. Due to the inherent chemical detoxification mechanism of microbes and energy-dependent ion efflux by the membrane proteins of the cell, which functions either as the proton or chemiosmotic cation or ATPase anti transporters, microbes show resistance to toxic heavy metals. Changes in solubility are also a major point that play role in microbial resistance. Hence, the microbes play the role of detoxification of metal ions via precipitation or reduction to insoluble non-toxic metal nanoclusters from soluble toxic inorganic ions (Ogi et al. 2011). This microbial detoxification can be accomplished via intracellular bioaccumulation or precipitation or extracellular biosorption, biomineralization, complexation (Hansda et al. 2016). However, extracellular synthesis has a wide range of commercial applications. In such a biological process, optimization of monodispersed conditions should be precisely followed due to the major concern of polydispersity; formation of less polydispersed and accumulated particles of specific dimensions are found in intracellular synthesis (Giovagnoli et al. 2014; Bharathi et al. 2020). This microbial synthesis can be performed via various microbes such as algae, fungi, bacteria, and yeast and are discussed in this section.

4.1 Algae

Green synthesis of various nanoparticles via algae has been explored by many researchers which are summarized in Table 1. Green synthesis of Ag NPs via freshwater *Chara vulgaris* algae was explored by Hassan *et al.* (Hassan et al. 2021a). They initially dried the algae and ground it to form the 100-ppm aqueous extract followed by the addition of $AgNO_3$ in various ratios but the perfect synthesized nanoparticles of Ag were accessible by the ratio 3:1 of *Chara vulgaris*: $AgNO_3$; the size of these nanoparticles being 16.99 ± 0.3 nm by SEM imaging. These Ag NPs showed immense potential against bacteria *Pseudomonas aeruginosa, Klebsiella pneumonia, and Escherichia coli*. The complete process of synthesis and antibacterial activity is provided in Figure 6. Other than these, various algae have been used for nanoparticles synthesis such as *Bifurcaria bifurcate, Caulerpa peltate, Hypnea Valencia, Sargassum myriocystum, Sargassum muticum, Sargassum muticum*, etc. (AlNadhari et al. 2021).

Table 1
Green synthesis of nanoparticles using Algae.

Year	Nanoparticle	Name of Algae	Size (nm)	Morphology	Application	References
2021	Ag	*Amphiroa rigida*	25	Spherical	Antibacterial and anticancer	(Gopu et al. 2021)
2021	Ag	*Chara vulgaris*	16.99 ± 0.3	-	Antibacterial	(Hassan et al. 2021a)
2021	ZnO	*Tetraselmis indica*	20-40	Spherical	Textile, cosmetic, biomedical, and food packaging	(Thirumoorthy et al. 2021)
2021	Pd	Padina boryana	11.16	Crystal	Nano drug against multidrug-resistant bacteria and cancer cells	(Sonbol et al. 2021)
2021	CuO	*Macrocystis pyrifera*	2-50	Spherical	-	(Araya-Castro et al. 2021)
2020	ZnO	*Anabaena cylindrica*	40-60	Rod shape	Antimicrobial	(Bhattacharya et al. 2020)
2020	Au and Ag	*Neodesmus pupukensis*	5-34	Circular	Antioxidant and antimicrobial	(Omomowo et al. 2020)
2020	Ag	*Portieria hornemannii*	-	Spherical	Antibiotics in the treatment of fish diseases	(Fatima et al. 2020)
2020	Ag	*Chlorella ellipsoidea*	220.8 ± 31.3	Spherical	Degradation of MB and MO, Antibacterial	(Borah et al. 2020)
2020	Ag	*Noctiluca scintillans*	4.5	Spherical	Anticancer and antibacterial	(Elgamouz et al. 2020)
2019	Ag	*Botryococcus braunii*	40-90	Spherical, cubical and truncated triangular	Catalyst in synthesis of benzimidazoles	(Arya et al. 2019)
2019	Ag	*Ulva armoricana*	12.5	Spherical	Antibacterial	(Massironi et al. 2019)
2019	Au	*Gelidiella acerosa*	5.81-117.59	Spherical	alpha glucosidase enzyme inhibition, antibacterial, antioxidant	(Facile green synthesis of gold nanoparticles from marine algae Gelidiella acerosa and evaluation of its biological PotentialSenthilkumar et al. 2019)
2019	Ag	*Chlorella vulgaris*	40-90	Spherical, cubical and truncated triangular	Catalyst for the synthesis of quinolines	(Mahajan et al. 2019)

Year	Nanoparticle	Name of Algae	Size (nm)	Morphology	Application	References
2019	ZnO	*Cladophora glomerata*	14.39 - 37.85	Spherical	Biomedical	(Abdulwahid et al. 2019)
2018	Cu and Ag	*Botryococcus braunii*	10-70 (Cu), and 40-100 (Ag)	Cubical and spherical (Cu), spherical, cubical, and truncated triangular (Ag)	Antimicrobial	(Arya et al. 2018)
2018	Ag	*Laminaria japonica*	20-30	Oval	Seed treatment, pharmacy	(Kim et al. 2018)
2018	Ag	*Sargassum wightii*	18.45–41.59	Spherical	Pharmacological agent	(Deepak et al. 2018)
2018	Au	*Sargassum crassifolium*	5-300	Varied	Biomedical	(Maceda et al. 2018)
2017	Pd	*Chlorella vulgaris*	15	Spherical	-	(Arsiya et al. 2017)
2017	CdS	*Chlamydomonas reinhardtii*	2-7	Spherical	Photodegradation of organic dyes	(Rao and Pennathur 2017)
2017	Ag	*Spyridia fusiformis*	5-50	Spherical and rounded Rectangle	Antibacterial	(Murugesan et al. 2017)
2017	Au	*Cystoseira baccata*	8.4 ± 2.2	Spherical	Anticancer	(González-Ballesteros et al. 2017)

4.2 Fungi

The synthesis of nanoparticles by fungi has more advantages as compared to other microorganisms (Dhillon et al. 2012). As compared to bacteria and plants, the fungal mycelial mesh can withstand agitation, flow pressure, and some other conditions in chambers and bioreactors, being easy to grow, handle, and fabricate. Fungi have outstanding metal bioaccumulation capacity with high binding capacity, their tolerance, and some other conditions such as intracellular uptake which is facile to handle under research conditions (Yadav et al. 2015). Nowadays, many fungi are in demand for nanoparticle synthesis such as *Volvariella volvaceae* (Bedlovicová and Salayová 2017), *Trichothecium sp.* (Qu et al. 2019), *Aspergillus fumigatus* (Vasanthi bathrinarayanan et al. 2013), *Penicillium brevicompactum* (Shaligram et al. 2009), *Aspergillus niger* (Soni and Prakash 2012), *Colletotrichum sp.* (Suryavanshi et al. 2017), *Fusarium semitectum* (Basavaraja et al. 2008), *Phoma glomerata* (Gade et al. 2014), *Penicillium fellutanum* (Kathiresan et al. 2009; Chandrappa et al. 2016), *Phaenerochaete chrysosporium* (Laxmi Sharma et al. 2021), *Fusarium oxysporum* (Gupta and Chundawat 2020), *Cladosporium cladosporioides* (Manjunath Hulikere and Joshi 2019).

Kaplan *et al.*(Kaplan et al. 2021) synthesized Ag NPs with the extract of *Boletus edulis* and *Coriolus versicolor.* They dried and pulverized these with a laboratory blender and 5 g of that powdered mushroom in 50 mL of distilled water was heated for 90 minutes at 60°C followed by filtration and centrifugation (10 minutes at 6000 rpm) of mushroom extract and stored at 4°C. For the synthesis of nanoparticles, 25 mL of mushroom extract were allowed to react in the microwave (MW) for 2 minutes at 475 W with $AgNO_3$ solution (25 mL of 10 mM) while pH was adjusted to 12 for

Coriolus versicolor and 10 for *Boletus edulis* using NaOH. After that, the mixture was kept for centrifugation (20000 g for 10 min), the nanoparticles designated BE-Ag NPs and CV-Ag NPs were precipitated which were washed several times and dried for further use (Figure 7) (Kaplan et al. 2021). Recently synthesized several nanoparticles by adopting green routes via fungi are summarized in Table 2.

Table 2
Green synthesis of nanoparticles using Fungi

Year	Nanoparticle	Name of Fungi	Size (nm)	Morphology	Application	References
2021	ZnO	*Phanerochaete chrysosporium*	50	Hexagones	Antimicrobial	(Sharma et al. 2021)
2021	Ag	*Trichoderma harzianum*	21.49	Cubes	Antioxidant, antibacterial	(Konappa et al. 2021)
2021	MgO	*Rhizopus oryzae*	20.38 ± 9.9	Spherical	Antimicrobial, mosquitocidal action, and tanning effluent treatment	(Hassan et al. 2021b)
2021	Au	*Morchella esculenta*	16.51	-	Biomedical	(Acay 2021)
2021	Ag	*Aspergillus sydowii*	1-24	Spherical	Antifungal and antiproliferative activity to HeLa cells	(Wang et al. 2021)
2020	Cu	*Aspergillus flavus*	2-60	Spherical	Biomedical	(Saitawadekar and Kakde 2020)
2020	Ag	*Ganoderma lucidum*	15-22	Spherical	Antimicrobial, antibacterial, antifungal	(Aygün et al. 2020)
2020	ZnO	*Periconium sp.*	16-78	Quasi spherical	Antimicrobial and antioxidant	(Ganesan et al. 2020)
2020	Au	*Fusarium solani*	40-45	Needle and flower like	Anticancer	(Clarance et al. 2020)
2020	Ag, CuO and ZnO	*Trichoderma harzianum*	5-18	Spherical	In biotechnological process	(Consolo et al. 2020)
2019	Au	*Fusarium oxysporum*	22-30	Spherical or hexagonal	Therapeutic	(Naimi-Shamel et al. 2019)
2019	SeS	*Saccharomyces cerevisiae*	5-7	Spherical	Antifungal	(Asghari-Paskiabi et al. 2019)
2019	Ag	*Pleurotus sp.*	2-100	Spherical	Biomedical field	(Owaid 2019)
2019	Fe_2O_3	*Trichoderma asperellum*	18-32	Spherical	Biomedical and waste water treatment	(Mahanty et al. 2019)
2018	Ag	Aspergillus fumigatus	1-50	Spherical	Antimicrobial	(Kalyani et al. 2018)
2018	Ag	*Aspergillus niger*	61	Spherical	Antimicrobial and anticancer	(Rayaman et al. 2018)
2018	MgO	*Penicillium chrysogenum*	5-12	Irregular	Antimicrobial	(El-Sayyad et al. 2018)
2018	Ag	*Pleurotus sajor-caju*	16.8	Spherical	Antifungal	(Musa et al. 2018)

Year	Nanoparticle	Name of Fungi	Size (nm)	Morphology	Application	References
2018	Au	*Pleurotus ostreatus*	2-20	Spherical	Biotechnological applications	(Vetchinkina et al. 2018)
2018	Ag	*Lenzites betulina*	14-50	Spherical	Antioxidant	(Sytu and Camacho 2018)
2017	Ag	*Pleurotus ostreatus*	40	Spherical	Antibacterial	(Al-Bahrani et al. 2017)
2017	Ag	*Aspergillus oryzae*	61.15±11.45	Triangular	Antimicrobial	(Silva et al. 2017)
2017	Au	*Aspergillum sp.*	50.3	Spheres, triangles, hexagons and irregular	Efficient catalysts for aromatic pollutants degradation	(Qu et al. 2017a)
2017	Ag	*Aspergillus niger*	41.9	Spherical	Antibacterial and antioxidant	(Hemashekhar et al. 2017)
2017	Au	*Mariannaea species*	37.4	Sphere, hexagon, and irregular shapes	Catalytic reduction of 4- NP	(Pei et al. 2017)
2017	Ag	*Aspergillus terreus*	16.54	Spherical	Antibacterial	(Rani et al. 2017)

4.3 Bacteria

Bacteria is one of the best candidates for reducing metal ions to nanoparticles with its unique abilities because of its high growth rate and ease of handling. Bacteria is easy to genetically mold and manipulate for metal ion's biomineralization as compared to other microbes (Liu et al. 2011; Vaseghi et al. 2018). Due to high exposure to continual toxic and harsh environmental conditions, generally, its surroundings have a high concentration of heavy metal ions. They have developed some natural defense mechanisms such as extracellular precipitation, intracellular sequestration, change in metal ion concentration, and efflux pumps, which enables them to survive these harsh and stressful conditions and these mechanisms have been fruitfully utilized by bacteria for nanoparticle's synthesis.

When some bacteria such as *Sulfolobus acidocaldarius, T. thiooxidans*, and *Thiobacillus ferrooxidans*, were grown on elemental sulfur as an energy source, they could reduce ferric ions to the ferrous state. *T. thiooxidans* was able to complete the reduction at low pH medium aerobically but it was unable to oxidize the ferrous ions again, hence the ferrous ions were stable to autooxidation. The bio reduction by *T. ferrooxidans* from ferric ions was not aerobic because the presence of oxygen boosts the bacterial reoxidation of ferrous ions (Brock and Gustafson 1976). Some bacteria used in nanoparticles synthesis are *Pseudomonas rhodesiae, Bacillus megaterium, Dietzia maris, Bacillus haynesii, Streptomyces sp., Actinomycetes sp.*, etc (Alsamhary 2020; Costa et al. 2020; Golińska 2020; Hamed et al. 2020; Huq 2020a; Akintelu et al. 2021; Goel et al. 2021; Hashem et al. 2021). The other synthesized nanoparticles by various bacteria, their properties, and their applications are presented in Table 3.

Table 3
Green synthesis of nanoparticles using bacteria.

Year	Nanoparticle	Name of Bacteria	Size (nm)	Morphology	Application	References
2021	ZrO	*Enterobacter sp.*	33 - 75	Spherical	Antifungal activity against bayberry twig blight disease	(Ahmed et al. 2021)
2021	Ag	*Bacillus subtilis*	2–26	Spherical	Antibacterial	(Yu et al. 2021)
2021	TiO_2	*Achrmobacter sp.*	5-10	Irregular	Antimicrobial	(Farag et al. 2021)
2021	Ag	*Dietzia maris*	40-50	Spherical	Wound healing activity	(Venil et al. 2021a)
2020	Ag	*Pseudomonas sp.and Enterobacter*	63.50 and 45.81		Antibacterial	(Saleh 2020)
2020	Ag	*Bacillus amyloliquefaciens*	1.23 -10.80	Spherical	Antimicrobial potential against phytopathogens	(Abd El Aty and Zohair 2020)
2020	Ag	*Serratia spp.*	-	-	-	(De Silva et al. 2020)
2020	Ag	*Pseudoduganella eburnea*	8-24	Spherical	Antimicrobial agent for various therapeutic applications.	(Huq 2020b)
2019	Au	*Bacillus licheniformis*	40	Irregular	Antimicrobial	(Scala et al. 2019)
2019	Ag	*Pseudomonas rhodesiae*	20-100	Spherical	Antibacterial	(Hossain et al. 2019)
2019	ZnO	*Bacillus haynesii*	20-100	Spherical	Medical and non-medical fields	(Rehman et al. 2019)
2018	Ag	*Nostoc linckia*	5-60	Spherical	Antibacterial	(Vanlalveni et al. 2018)
2018	ZnO	*Bacillus megaterium*	45-95	Rod and cube shape	Antibacterial and therapeutic agents	(Saravanan et al. 2018)
2017	Au	*Streptomyces griseoruber*	5-50	Spherical to triangular and hexagonal	Degradation of MB	(Ranjitha and Rai 2017)
2017	Ag	*Streptomyces genus*	160	Spherical	Nanomedicine and cosmetology industries.	(Silva-Vinhote et al. 2017)
2017	Ag	*Actinomycetes sp.*	-	-	Antibacterial	(Thomas 2017)
2017	Ag	*Streptomyces Sp.*	11-38	Spherical	Antibacterial	(Gupta et al. 2017)

4.4 Yeast

Yeast extract plays the role of reducing and capping agent which encompasses carbohydrates, vitamins, and amino acids whereas metal ions serve as an electron acceptor. The organic capping agents provide stability to the synthesized monodispersed nanoparticles and as a result, these can be preserved without precipitation for more than a year (Boroumand Moghaddam et al. 2015; Skalickova et al. 2017). The synthesis of Ag NPs using yeast has been described by Shu and coworkers with the formation of yeast micelles in yeast extract by self-assembly of biomolecules followed by reduction of Ag^{+} via *in situ* method which provided stabilization to the nanoparticles. Affinity to the bacterial membrane is enhanced by the coating of the surface of Ag NPs wherein the permeability of the cell wall also increased. When peptidoglycan interacted with Ag NPs, the change in configuration of peptidoglycan occurred which resulted from the damage of bacteria by the apoptosis process (Figure 8) (Shu et al. 2020). Yeast-assisted synthesis of nanoparticles and their applications are provided in Table 4.

Table 4
Green synthesis of nanoparticles using Yeast.

Year	Nanoparticle	Name of Yeast	Size (nm)	Morphology	Application	References
2021	Ag	*Saccharomyces cerevisiae*	6.72	Irregular	Antibacterial	(Elnagar et al. 2021)
2021	Au	*Candida parapsilosis*	-	-	The catalyst for aryl amines synthesis	(Krishnan et al. 2021)
2021	Ag	*Pichia kudriavzevii* HA-NY_2 *and Saccharomyces uvarum* HA-NY_3	20.655 ± 9.48 (AgNPsK) and 2.4 ± 6.02 (AgNPsU)	Cubic (AgNPsK) and round (AgNPsU)	Anticancer	(Ammar et al. 2021)
2020	AgCl	*Commercial yeast*	9-51	Spherical	Anti-mycobacterial Activity	(Sivaraj et al. 2020)
2020	Ag	*Yeast extract*	13.8	Spherical	Disinfection of multidrug-resistant bacterial strains,	(Shu et al. 2020)
2019	Se	*Magnusiomyces ingens*	70-90	Spherical and quasi-spherical	Antibacterial	(Lian et al. 2019)
2019	Pd	*Ogataea polymorpha*	20-40	Spherical and hexagonal	Biosensors and fuel cell	(Gayda et al. 2019)
2019	Se	*Saccharomyces boulardii*	20 -240	Spherical	-	(Bartosiak et al. 2019)
2019	Ag	*Brewer's yeast*	-	Spherical	-	(Yantcheva et al. 2019)
2019	Au	*Yarrowia lipolytica*	104	Polygonal or spherical	Anticancer	(Ben Tahar et al. 2019)
2018	Ag	*Yeast extract peptone dextrose*	-	-	Antibacterial	(Daphne et al. 2018)
2018	Ag	*Saccharomyces cerevisiae*	10-60	Spherical	Antibacterial	(Sowbarnika et al. 2018)
2018	Ag	*Rhodotorula sp. strain* ATL72	8.8 – 21.4	spherical and oval	Antimicrobial	(Soliman et al. 2018)
2018	Au and Ag	*Phaffia rhodozyma*	2.22±0.7 (Au) and 4.1±1.44 (Ag)	Spherical (Au) and Quasi spherical (Ag)	Antifungal	(Rónavári et al. 2018)
2018	Ag	*Meyerozyma guilliermondii* KX008616	2.5–30	Spherical	Biomedical	(Alamri et al. 2018)
2018	Au	*Magnusiomyces ingens* LH-F1	20.3	Uniform	Catalysts in reduction of organic contaminants.	(Qu et al. 2018)

Year	Nanoparticle	Name of Yeast	Size (nm)	Morphology	Application	References
2018	TiO_2	*Baker's yeast*	6.7 ± 2.2	Spherical	Antimicrobial	(Peiris et al. 2018)
2018	Ag	*Candida albicans*	2.0 - 7.3	Spherical	Antimicrobial	(Ananthi et al. 2018)
2017	Au	*Saccharomyces cerevisiae*	9.99 ± 1.63	Spherical	High catalytic dechlorination efficiency	(Shi et al. 2017)
2017	ZnO and Ag	*Marine yeast*	86.27 (ZnO) and 31.78 (Ag)	Round for both	Antioxidant	(Aswathy et al. 2017)
2017	Se and Ag	*Pichia pastoris*	70-180	Spherical	Biomedical	(Elahian et al. 2017)
2017	Ag	*Rhodotorula mucilaginosa.*	11.0	Spherical		(Salvadori et al. 2017)
2017	ZnO	*Pichia kudriavzevii*	10–61	Hexagonal	Antimicrobial and antioxidant	(Moghaddam et al. 2017)

5. Types Of Nanoparticles Produced Using Microorganisms

5.1 Ag nanoparticles (Ag NPs):

In old human civilizations, people used silver and silver salts, but in recent years the fabrication of Ag NPs has been in demand due to their outstanding applications in the biomedical field such as antifungal, antibacterial, antioxidants as well as in agricultural and environmental remediation. These nanoparticles hold a specific place among other metals used in the biomedical field and have been widely explored via the greener routes (Jadoun and Dilfi 2021; Uthaman et al. 2021). Gevorgyan *et al.*(Gevorgyan et al. 2021) reported the use of Ag NPs as an excellent inhibitor of Gram-positive *S. aureus* and Gram-negative *S. typhimurium.* The synthesis, properties and applications of Ag NPs produced by microbes are discussed in this section.

5.1.1 Synthesis and properties

Synthesis of Ag NPs using the bacteria, *Bacillus Licheniformis*, as a reducing and capping agent was performed by adding the aqueous solution of silver nitrate ($AgNO_3$) to the biomass of bacterial extract; the size of nanoparticles being 50 nm, (Figure 8a).(Kalimuthu et al. 2008) The culture supernatants of *Staphylococcus aureus* were also used for Ag NPs synthesis (Nanda and Saravanan 2009). Fungi have been used to procure Ag NPs where the reduction of $AgNO_3$ was performed by the enormous amount of enzymes secreted by fungi and was further characterized and deployed in antimicrobial, antiviral, and wound dressing activities (Khan et al. 2018) (Figure 8b). The same nanoparticles were synthesized by using 5 mL of *Botryococcus braunii* algal extract when mixed with 1mM $AgNO_3$ and stirred. After saturation of reaction, the mixture was centrifuged for 20 minutes and the pellets were separated with supernatant. The obtained nanoparticles were washed several times and dried at 55°C for 5 hours (Figure 9c).

Enterobacteriaceae sp. bacteria were found useful for the quick synthesis of Ag NPs as Ashraf *et al.* (Ashraf et al. 2020) described their preparation using *Enterobacter cloacae* (SMP1) bacteria's cell-free supernatant protein. The bacterial strain was inoculated in liquid Luria Bertani (LB) broth for incubation in a rotatory shaker at 120 rpm at 37°C overnight followed by the centrifugation of the same at 600 g for 15 minutes. The supernatant was collected by

filtering it with 0.22 µm pore size filter paper and stored at 4°C and the pellet was discarded. For the synthesis of nanoparticles, 1.5 mM $AgNO_3$ solution was added to 1% of cell-free culture supernatant, and the analysis of the samples was done for 6 days. In different time intervals after 24 hours, the harvesting of aliquots of 200 µL sample was performed. For confirmation of synthesis, UV-Visible spectroscopy was used which indicated the reduction of silver ions (Ag^+) to zero-valent silver (Ag^0) affirmed by the typical peak of Ag found at 450 nm, (Figure 10i). The TEM micrographs revealed spherical shape and 10-20 nm size of nanoparticles while X-ray diffraction pattern and elemental mapping suggested crystalline nature and showed the presence of four elements (Figure 10ii).

Santos *et al.* (Santos et al. 2021) adopted an extracellular biosynthetic route for Ag NPs assembly using 10 g of Entomopathogenic Fungi Biomass after the addition of deionized water (100 mL). The solution was incubated for three days at 25°C on a rotatory shaker at 100 rpm. Subsequently, the biomass was filtered and stored at 4°C for the synthesis of Ag nanoparticles. Afterward, 1 mL and 10 mM solution of an aqueous solution of $AgNO_3$ was added with 9 mL of fungal extract biomass and the solution was magnetically stirred, away from the sunlight, for 72 hours at 25°C. The mean diameter of obtained nanoparticles was found between 40.14 and 289.13 nm using DLS. Recently, the spherical nanoparticles of Ag were obtained by using *Trichoderma harzianum* (Guilger-Casagrande et al. 2021) while 10-30 nm of spherical shaped nanoparticles with little agglomeration could be obtained by *Bjerkandera sp.* R1 white-rot fungus (Osorio-Echavarría et al. 2021).

Kashyap *et al.* (Kashyap et al. 2021) adopted an intracellular green synthetic route using the microalgae *Scenedesmus sp*. for Ag/AgCl nanohybrids synthesis. The 0.5 and 1 mM of $AgNO_3$ precursor with extract of *Scenedesmus sp.* as a reducing agent was used for the synthesis; spherical particles with 10–20 nm and 10–50 nm in size were obtained, respectively. The change from transparent to deep brown color of the mixture of $AgNO_3$ and bacterial strain solution indicated the formation of hexagonal shaped Ag nanoparticles using *Bacillus anthracis* $PAFB_{2,}$ showed 0.428 with −15.5 mV Zeta potential value for polydispersity (PDI) index which indicated their good colloidal nature and long-term stability of nanoparticles; the size of nanoparticles being ~ 84 nm by AFM analysis (Banerjee et al. 2021). Some spherical shapes, ranging between 13–27 nm, were synthesized using *Paenarthrobacter nicotinovorans* MAHUQ-43 bacterial strain (Huq and Akter 2021).

5.1.2 Applications

Ag NPs synthesized using *Bacillus subtilis* were studied against five strains of multidrug-resistant microbes such as *Candida albicans*, *Klebsiella. Pneumoniae, Staphylococcus epidermidis, Staphylococcus aureus, and Escherichia coli*. The rate of MICs (minimum inhibitory concentrations) versus the clinical isolates revealed outstanding antimicrobial efficiency and revealed 100, 180, 200, 230, 300 μgmL^{-1} for *Candida albicans*, *Staphylococcus epidermidis, Escherichia coli*, *Staphylococcus aureus, and Klebsiella pneumonia*, respectively. Ag NPs were indicated to be toxic for gram-positive and gram-negative bacteria. These nanoparticles showed high antifungal activity and could be used to treat multidrug-resistant microorganisms (Alsamhary 2020). Ag NPs synthesized from green algae showed excellent catalytic activity in the reduction of 2-nitroaniline. The reduction of 2-nitroaniline (100 mg, 0.724 mmol) into *o*-phenylenediamine was performed in presence of Ag NPs (0.10 mg, 10% w/w of 2-nitroaniline) and sodium borohydride (60 mg). The reaction mixture was adjusted to ∼6 pH using glacial acetic acid for the removal of sodium borohydride. The product was further cyclized for 10-12 hours at 80°C with substituted aldehydes (0.724 mmol) to produce 2-aryl benzimidazoles (Figure 11a) (Arya et al. 2019). On the other hand, Ag NPs synthesized from a new bacterial strain showed outstanding antibacterial effect against standard and multi-drug resistant strains according to the Clinical and Laboratory Standards Institute (CLSI) which were analyzed using a good diffusion method. The nanoparticles inhibited the growth of the strains including *Salmonella paratyphi, Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis*

(ATCC 6633), *Escherichia coli* (ATCC 10536), *Shigella dysenteriae* (PTCC 1188), *Proteus vulgaris* (PTCC 1182), and *Klebsiella pneumonia* (ATCC 10031) around the wells, Figure 11 (b) (Nazari and Jookar Kashi 2021).

Ag NPs synthesized using *Anabaena variabilis* revealed antioxidant activity and antimicrobial activity (Ahamad et al. 2021). The IC_{50} value found in the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity was 13.22 ± 1.25 μg mL^{-1} while in the case of Ag nanoparticles synthesized by *Ecklonia cava* it was found 198 μg mL^{-1}. These nanoparticles were used in anticancer activity (Venkatesan et al. 2016). In the case of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Ag NPs synthesized using *Acutodesmus dimorphus*, revealed an IC_{50} value of 14.41 μg mL^{-1} while using *Anabaena variabilis, it was 2*.67 ± 0.5 μg mL^{-1} (Chokshi et al. 2016). The antimicrobial activity against *Bacillus amyloliquefaciens, Streptococcus pyogenes, Staphylococcus aureus*, and *Salmonella* was analyzed by disc diffusion method and after incubation of 24 hours, the difference in the zone of inhibition was observed between control samples and green synthesized nanoparticles treated with microorganisms (Ghiuta et al. 2021).

Lactobacillus bulgaricus mediated Ag NPs showed antibacterial activity against *Salmonella typhi*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*, which revealed 17-mm mean values of zone inhibition for the *Salmonella typhi* and *Staphylococcus epidermidis* while 15-mm for *Staphylococcus aureus*. The antibiotics effects were studied according to Birmingham Children's Hospital guidelines (2014); antibiotic activities were varying against selected bacterial strains resulted in sensitivity to antibacterial activity in comparison of antioxidant activity (Naseer et al. 2021). Recently, The Ag NPs fabricated using *Cedecea sp.* showed immense potential in antibiofilm activity. These Ag NPs unveiled strong MIC and MBC (minimum bactericidal concentration) values against *E. coli* (12.5 μg/μl and 12.5 μg/mL) and *P. aeruginosa* 6.25 μg/μl and 12.5 μg/mL), respectively and were extremely stable for more than one year with strong antibacterial activity against biofilms of *P. aeruginosa* and *E. coli.* (Singh et al. 2021). Ag NPsacquired via extracellular synthesis using *Gloeophyllum striatum* were antibacterial. The cytotoxic and hemolytic activity was checked towards mammalian cells which revealed that after 24 hours, more than 30 μM triggered 50% hemolysis of RBC, and no toxicity was found at 0.5–10 μM concentrations, IC_{50} value at 24 hours being 28.76 μM. For the ecotoxicity study, the aquatic crustaceans *Artemia franciscana* and *Daphnia magna* were selected. In the saline ecosystem, *Artemia franciscana* showed higher tolerance than *Daphnia magna* towards Ag nanoparticles. The EC_{50} values for *Daphnia magna* and *Artemia franciscana* were found to be 0.275 and 61.97 μM, respectively (Zawadzka et al. 2021).

5.2 Au nanoparticles (Au NPs)

Gold nanoparticles (Au NPs) are the topic of interest and received much attention due to their simple synthesis and extensive applications. Initially, Beveridge and Murray used *Bacillus subtilis* for the synthesis of octahedral 5-25 nm-sized Au nanoparticles (Beveridge and Murray 1980). Au NPs have been used as therapeutics, disease diagnostic materials, biocatalysts, and nanomedicine. Biocompatibility is the major concern for use in nanomedicine, hence, adopting green synthesis via microbes is an alternative to achieve this objective (Aminabad et al. 2019; Nejati et al. 2021); greener synthesis and the applications are discussed in this section.

5.2.1 Synthesis and properties

Synthesis of Au NPs using extract of *Gelidiella acerosa* marine algae (10 mL) was completed by mixing the algal extract with the aqueous solution of $HAuCl_4 \cdot 3H_2O$ (gold chloride; 90 mL of 1 mM) and keeping the mixture under the static condition at 37°C. The Au NPs were washed and centrifuged for 15 minutes at 10,000 rpm to separate the nanoparticles which were dried at 50°C and kept at 4°C for further characterization and applications; crystalline nanoparticles had a size between 5.81 nm to 117.59 with spherical and hexagonal shapes. These Au NPs were found

outstanding against inhibition of α- glucosidase and α-amylase enzyme with 2.8 ± 0.02, 4.1 ± 0.01 and 2.1 ± 0.01, 3.7 ± 0.01 µg/mL, respectively, Figure 12 (Senthilkumar et al. 2019).

Clarance *et al*. (Clarance et al. 2020) fabricated Au NPs using extract of *Fusarium solani.* They cultured the fungi in YEPD (yeast extract peptone dextrose) broth, and the culture was incubated at 28°C in the shaker at 120 rpm and kept for 9 days for further incubation. After that, it was filtered with cheesecloth and washed multiple times. Sterile Milli Q water (100 mL) was added to that biomass and kept undisturbed for 2 days. Further, it was filtered through Whatman No-1 filter paper and maintained the pH at 8.5 with 0.1 N NaOH. $HAuCl_4$ (99 mL of 1 mM) solution was added to the above fungal extract (1.0 mL) and kept for incubation under dark for 48 hours. Formation of pink-ruby red color indicated the formation of nanoparticles of Au and the peak between 510 and 560 nm was observed for plasmon band while the peaks at 1413 cm^{-1} in FTIR attributed to the amine II bands of protein. The nanoparticles were flower-like and needles shaped with 40-45 nm size.

Marine microbe (*Vibrio alginolyticus*) was used for the synthesis of Au NPs adopting the extracellular synthesis route. The culture was inoculated and incubated for 24 hours at 40°C on an orbital shaker at 120 rpm. After that, the mixture was centrifuged for 15 minutes at 8000 rpm and the supernatant was collected followed by the addition of aqueous HAuCl (chloroauric acid) (1mM) and again incubated under the same conditions. The nanoparticles were precipitated out and were separated by centrifugation, washed, and dried to achieve powder of nanoparticles. The 50-100 nm irregular monodispersed nanoparticles were suggested by TEM analysis (Shunmugam et al. 2021). *Sargassum wightii*, a marine alga was used for the extracellular synthesis of 8 to 12 nm-sized monodisperse Au NPs and a peak at 527 nm in the UV-visible spectrum suggested the plasmon absorbance of Au nanoparticles (Singaravelu et al. 2007). The same method was adopted by *Mukherjee et al.*(Mukherjee et al. 2002) for the fabrication of nanoparticles using fungus *Fusarium oxysporum* by exposing aqueous $AuCl_4^-$ ions to the fungus extract. Three different fungi, *Fusarium oxysporum*, *Fusarium* sp., and *Aureobasidium pullulans* were used for the synthesis of Au NPs via mixing the fungal strain cells with $AuCl_4^-$ ions solution (Zhang et al. 2011).

Salouti *et al.* (Zonooz et al. 2012) used *Streptomyces sp.* ERI-3 for the synthesis of Au NPs. The culture was incubated at 200 rpm for 48 hours at 28°C and $HAuCl_4$ solution (50 mL of 1 mM) was added to the supernatant (10 mL) and kept on an orbital shaker at the same aforementioned conditions. TEM studies suggested spherical and cylindrical-shaped nanoparticles ranging in between 80-200 nm. The synthesis was optimized with different reaction conditions and the best optimum conditions were found to be, pH 6, incubation time 12-hours, temperature: 30°C, and $HAuCl_4$ concentration 3 mM. *Cladosporium cladosporioides* (marine endophytic fungus) were used for 60 nm average-sized Au NPs synthesis and showed noteworthy antioxidant activity compared to ascorbic acid (M et al. 2017). Au NPs have been synthesized using various bacteria such as *Bacillus subtilis*, *Shewanella algae*, *Pseudomonas aeroginosa*, *Escherichia coli*, *Lactobacillus* sp., *Thermomonospora* sp., and *Rhodococcus* sp (Ahmad et al. 2003a, b; Mandal et al. 2006; He et al. 2007; Moghaddam 2010).

5.2.2 Applications

The organic solvent-free nature and high photocatalytic dechlorination have been accomplished by the Au NPs synthesized using *Saccharomyces cerevisiae* yeast. These 9.99 ± 1.63 nm-sized mono-dispersed Au NPs revealed the conversion of quinclorac to 8-quinoline-carboxylic acid by dechlorination using sodium borohydride which followed pseudo-first-order kinetics. (Figure 13a) (Shi et al. 2017). Qu *et al.* (Qu et al. 2017b) synthesized Au NPs from *Trichoderma sp*. for their potential application in azo dyes decolorization in contaminated water; these dyes absorb and reflect the sunlight which affects the aquatic organism's growth as well as the photosynthesis process. The above nanoparticles could decolorize the Acid Brilliant Scarlet GR up to 94.7% in 120 minutes (Figure 13b) at various

concentrations of dye; at a dye concentration of 25 mg/L, degradation was more than 90% in 40 minutes while at 50 mg/L, 90% of decolorization was observed in 100 minutes (Qu et al. 2017b).

Psychrotolerant Antarctic mediated synthesis of Au NPs showed antimicrobial activity against sulfate-reducing bacteria (*Desulfovibrio desulfuricans*) which was assessed by the optical density of bacteria culture. The nanoparticles reduced the *Desulfovibrio desulfuricans* numbers to 12% (10^6 to 10^3cells mL^{-1}) along with the reduction of sulphate reducing activity to 7% (0.0246 nanomoles mL^{-1} day^{-1} to 0.0016 nanomole mL^{-1} day^{-1}); MIC was calculated to be 200 μg mL^{-1} concentrations. The nanoparticles revealed the antimicrobial activity against *Bacillus, Pseudomonas aeruginosa, Escherchia coli, Klebsiella pneumonia, and Staphylococcus aureus.* The Au NPs deteriorate the cells of microbes by connecting with the surface, creating an aperture in the cell wall which induces seeping of cell contents resulting in death. They could inhibit the transcription by inhibiting the DNA (Shunmugam et al. 2021).

The Au NPs showed immense potential in anticancer activity which was assessed against colon cancer cell line by a dose-dependent inhibition activity. These nanoparticles were synthesized as a chemotherapeutic alternative to escape from drugs that are toxic and exhibit numerous side effects in the body; IC_{50} value was found to be 15 μg/mL by comparing it with standard (Figure 14a). The morphological analysis revealed a cytotoxicity effect on the HCA-7 (human colon cancer) cell line when treated with Au NPs. There was a clear difference seen in treated and untreated cell organelles and unveiled noteworthy cell damage by Au NPs with undistinguished cell debris which indicated a significant contribution of these biogenic Au NPs for human colon cancer cells (Figure 14b) (Shunmugam et al. 2021). *Ecklonia cava*, a marine alga mediated spherical (20-50 nm size) Au NPs showed antimicrobial activity against some pathogenic organisms and revealed the diameter of the zone of inhibition (20 μL) for *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 1015, *Bacillus subtilis* ATCC 6633, *Aspergillus fumigates* ATCC 1022, *Escherichia coli* ATCC 10536, *Aspergillus brasiliensis* ATCC 16404, *Staphylococcus aureus* ATCC 6538 and, *Pseudomonas aeruginosa* ATCC 27853 about 23.3 ± 0.25, 24.6 ± 0.23, 19.7 ± 0.21, 21.5 ± 0.25, 31.8 ± 0.32, 19.3 ± 0.26, 16.6 ± 0.30 and 21.3 ± 0.28 mm, respectively. The highest antimicrobial activity was shown against *Aspergillus niger* ATCC 1015 and *Escherichia coli* ATCC 10536 (Venkatesan et al. 2014). *Sargassum incisifolium* (brown algae)-assisted secured Au NPs were studied for antimicrobial and anticancer activity (Mmola et al. 2016).

5.3 Pt nanoparticles (Pt NPs)

Platinum is a rare metal, used in cancer treatments, fuel cells, or catalytic converters. Its enormously low abundance makes it the topic of immense interest due to its unique structural, catalytic, and optical properties with huge potential in biomedical applications and catalysis (Yamada et al. 2015; Siddiqi and Husen 2016; Pedone et al. 2017).

5.3.1 Synthesis and properties

The cultivation of the control yeast and hydrogenase-displaying yeasts was performed anaerobically in AHLU + SDC medium (synthetic dextrose medium). The centrifugation of the cells was accomplished in 5 minutes at 3000×*g.* and incubated in 7.4 pH PBS comprising 100 μM $PtCl_4^{2-}$ salt. The mixture was allowed to react anaerobically at a rotatory shaker at 37°C for 72 hours and the black precipitate was separated from the solution and reduced Pt NPs were collected.(Ito et al. 2016) Green synthesis of Pt NPs has been reviewed by Puja *et al.* (Puja and Kumar 2019) wherein the syntheses are described by various biological species using H_2PtCl_6 as a precursor and the indication of nanoparticles synthesis by a color change and their potential application in biomedical fields (Figure 15a). *Fusarium oxysporum* was incubated with H_2PtCl_6 for extracellular synthesis of stable Pt NPs of 5-30 nm size (Syed and Ahmad 2012). The same fungi were used by Govender *et al.*(Govender et al. 2009) by isolating 10 mL of 120 nmol min^{-1} mL^{-1} purified hydrogenase and reacting with 10 mL of 1 mM solution of $PtCl_2$ or H_2PtCl_6 under the hydrogen atmosphere

and optimized temperature (38°C) and pH (7.5). For hydrogenase reactions, these conditions were found optimum as after 2 hours 30% reduction of $PtCl_2$ was observed, after 4 hours 70% and after 8 hours 90% reduction was noticed although for H_2PtCl_6, these results didn't match, after 8 hours also, 96% platinum salt was remaining. It was concluded that during the redox mechanism, H_2PtCl_6 functioned as an electron acceptor and indicated that the enzymes and metal/metal ions transferred electrons directly. Some hydrophobic active channels existed in between the molecular surfaces and the active site which were used as the passage by metal ions having 0.45-0.60 nm diameter. It was assumed that these channels were not small for $PtCl_2$ but were indeed very small for H_2PtCl_6 (Figure 15b).

The culture of *Neurospora crassa* extract was kept for 5 to 10 days at 28°C to attain macroconidia and harvested at 4°C in glycerol for further use, 100 mL of potato dextrose broth was inoculated with 100 µl of macroconidia for obtaining its biomass. Subsequently, the steps of incubation and filtration were performed at optimized temperature and time for starting the reduction process. The absorption spectra of both the control and Pt NPs were performed in which a peak at 530 nm was obtained for Pt NPs with quasi-spherical shape and 4-35 size by HRTEM analysis. From dark-field images, the size distribution of nanoparticles showed a total of 234 nanoparticles in which 60% of particles were between 40 to 50 nm in size and more than 70 nm size was found only for 2% nanoparticles (Figure 14c) (Castro-Longoria et al. 2012) *Acinetobacter calcoaceticus* mediated synthesis of Pt NPs was performed by Gaidhani *et al.* (Gaidhani et al. 2014) where they described the reduction of H_2PtCl_6. The entrenchment of nanoparticles within the cells was seen by AFM analysis and average surface roughness was found to change when compared to control cells which indicated the formation of Pt NPs. The size of nanoparticles was 2-3.5 nm while the shape was found cuboidal by HRTEM analysis.

5.3.2 Applications

The nanoparticles fabricated using *P. chrysogenum* were assessed for anticancer activity on myoblast C2C12 cancer cells. To evaluate the mitochondrial activity, death of cell and survival in presence of biogenic Pt NPs, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed as it is the most vigorous method for analysis of nanotoxicology which elucidated the response of cells to metal toxicity along with evidence for the death of the cell, metabolic activities, and survival. The Pt NPs revealed a significant decrease in cell viability and mitochondrial reduction (90.4%) at 100 µg concentration while the control cell unveiled no mitochondrial reduction with maximum cell viability; nanoparticles of size less than 20 nm showed no cytotoxic against cancer cells. With the pre-treatment by Pt NPs, cell viability was reduced in human squamous carcinoma and A431 at 24 hours; IC_{50} values were found to be 41.09%, (Subramaniyan et al. 2018). The Pt NPs were synthesized using various bacterial strains such as *Pseudomonas kunmingensis, Psychrobacter faecalis, Vibrio fischeri, Jeotgalicoccus coquina, Sporosarcina psychrophile, Kocuria rosea, Pseudomonas putida, Rhodotorula mucilaginosa*which exhibited antioxidant activity against the DPPH radical scavenging assay. The purple color of DPPH radicals changed to pale yellow by interacting with nanoparticles as Pt NPs provide transferability of electrons/hydrogen atoms to neutralize DPPH. It was a dose-dependent activity as the inhibition percent increased with the increment of Pt concentration. The degree of antioxidant activity of 1000 µg/mL nanoparticles were ordered from lowest to highest was MN23 < ADR19< KT2440 < KC19 < B-11177 < CCV1 < FZC6 < ZC15 (Figure 16) (Eramabadi et al. 2020).

5.4 Pd nanoparticles (Pd NPs)

Palladium is a very precious metal and is endowed with significant thermal and chemical stability, optical and electronic properties, with ease of biofunctionalization for enhancing their medical applications (Fahmy et al. 2020).

5.4.1 Synthesis and properties

Biogenic Pd NPs fabricated using green alga, *Botryococcus braunii* revealed the formation of 4.89 nm-sized and truncated triangular, spherical, cubical shaped nanoparticles which were synthesized by stirring the solution of algal extract (20 mL) and an aqueous solution of Pd $(OAc)_2$ (80 mL of 1 mM) for 3 hours at 60°C while maintaining pH in the range 6-7. A positive (with alga extract and Pd $(OAc)_2$) and negative control (without alga extract) were maintained for the comparison. The solution bearing Pd NPs was centrifuged for 30 minutes and washed for the removal of impurities. For better separation of nanoparticles, the process was repeated 3 times and nanoparticles were dried at 70°C in a hot air oven, the entire process of Pd NPs synthesis is depicted in Figure 17 (Anju et al. 2020).

Genetically modified *Pichia pastoris* fungus was found as the factory for producing Pd NPs. Parental and modified species were cultivated in YPD media (Yeast, Peptone, and Dextrose in 1,2,2% w/v) and inoculated with the covering of the flask by cheese cloth (two-folded) for oxygen transfer; 0.5% methanol was daily added to the flasks for AOX1 promoter induction. To reach optical cell density up to 0.1, the cells were cultivated in a shaker incubator at 30°C at 250 rpm. Subsequently, $PdCl_2$ was added to the flask to attain 60 mM final concentration and after the time intervals of 0-, 6-, 12-, 24-, 36-, 48-, 60- and 72-hour, 10 mL of aliquots were collected for recording the absorbance value at 600 nm. The nanoparticles were collected by centrifugation at 5000×*g* for 10 min and washed thoroughly. The maximum absorption in recombinant yeast was found at 79.79%. The best equation of Pd NPs was found $y = 177.78\times \mathrm{Ln}(t) - 233.23$ and $y = 6.87\times \mathrm{Ln}(t) + 49.91$, for the Pd biosorption capacity (mg/g) and formation yield (%) (Elahian et al. 2020).

5.4.2 Applications

Pd NPs are generally used as a homogenous and heterogeneous catalyst in many of the organic reactions such as Suzuki coupling reactions and Suzuki-Miyaura cross-coupling reactions (Liu et al. 2021; Sun et al. 2021), medical diagnosis (Zhuge et al. 2019), cancer treatments (Kang et al. 2018), drug delivery (Zhang et al. 2019), antimicrobial activities (Nasrollahzadeh et al. 2020a), antioxidants (Fahmy et al. 2020), and as nanocatalysts for dye degradation in effluents from textile industries among other biomedical applications (Gil et al. 2018; Pandey et al. 2021). *Escherichia coli* assisted obtained Pd NPs showed better catalytic activity to chemical counterparts at low temperature and in the air for the oxidation of benzyl alcohol; catalysis was performed with 180 mg of nanocatalyst and 50 mL of benzyl alcohol and the reactor was set to reach 90°C. These biogenic Pd NPs were compared with the chemically prepared catalyst in O_2 and noticed that at lower loadings of catalyst (6×10^{-5} mol l^{-1}), it displayed higher activities (Deplanche et al. 2012). Fahmy *et al.* (Fahmy et al. 2020) reviewed the Pd NPs synthesized adopting biological routes for their unique physiological properties and biomedical applications.

5.5 Cu nanoparticles (Cu NPs)

There are numerous types of copper (Cu) nanoparticles such as Cu, CuO, Cu_2O nanoparticles (Ighalo et al. 2021; Kumar et al. 2021; Medvedeva et al. 2021); they are widely known for their magnetic, optical, electric, and catalytic properties which are applicable in optoelectronics, photocatalysis, sensors, and biomedical fields such as antifungal, antibacterial, antioxidant, anticancer, antiviral, and drug delivery systems (Al-Hakkani 2020; Marouzi et al. 2021) .Their synthesis, properties, and applications are discussed in this section.

5.5.1 Synthesis and properties

The bacterial strains used for the nanoparticle's synthesis were inoculated in Luria–Bertani medium followed by incubation on a rotatory shaker at 200 rpm at 22°C. After 24 hours, the final concentration of 1 mM was attained with the addition of $CuSO_4 \cdot 5H_2O$. Then the incubation of the reaction mixture was performed further on a rotatory shaker at 150 rpm for 24-48 hours at 22°C. For control, heat-killed bacterial strains or without bacterial strains Luria–Bertani

medium with 1mM $CuSO_4$ was maintained. The color of the mixture changed to dark green from cyan blue suggesting the formation of Cu nanoparticles. When white-rot fungus *Stereum hirsutum* (Cuevas et al. 2015) and *Morganella* sp. (Lalitha et al. 2020) were used as reducing and capping agents, the same color change was observed affirming the formation of nanoparticles. The solution was further centrifuged at 5000 rpm for 20 minutes at 4°C and collected for washing with double distilled water. Short duration (15 seconds) ultrasonic wave irradiation was imparted for the recovery of nanoparticles from cell pellet and centrifuged for 20 minutes at 5000 rpm followed by recovery of the nanoparticles which were dried in an oven at 80°C; ovoidal or spherical and monodispersed nanoparticles ensued with 10-70 nm particle size and with an average size of 40 nm (John et al. 2021).

For the synthesis of Cu NPs using *Escherichia sp.*, the cultivation and incubation were accomplished in nutrient broth for 24 hours at 150 rpm followed by the addition of $CuSO_4$ (5 mM) and incubation again. The color change was noticed from bluish-green to dense green and it was phenotypic confirmation for the synthesis of Cu nanoparticles; in the UV region, it showed a peak at 325.89 affirming the synthesis of nanoparticles (Figure 18) (Noman et al. 2020). *Shewanella loihica* PV-4 mediated Cu NPs were synthesized by extracellular bioreduction of Cu (II) and the size of nanoparticles was found to be10–16 nm by TEM analysis while a strong Cu signal was observed by EDX (Energy-dispersive X-ray spectroscopy) analysis to confirm the synthesis (Lv et al. 2018).

5.4.2 Applications

Penicillium chrysogenum assisted spherical CuO NPs unveiled antibiofilm, antifungal, and antibacterial activity. The highest effect was shown by CuO NPs against *Staphylococcus aureus* at a concentration under MIC values. These nanoparticles could reduce the formation of biofilm by 68.8, 85.9, 94.4, 94.1 and 95% at concentrations 0.01, 0.03, 0.07, 0.15, and 0.3 mg/mL, respectively without any effect on bacterial growth (Figure 19a). However, they showed no effect on biofilm formation by *Pseudomonas aeruginosa* (Figure 19b). These nanoparticles showed antibacterial activity against Gram-positive and Gram-negative bacteria but the more effective diameter of clear zone was found against Gram-positive than Gram-negative bacteria. The formed clear zone diameters were 11.66 ± 0.33, 11.93 ± 0.52, 13.6 ± 0.4, 16.26 ± 0.63 and 22 ± 0.57 mm for *Salmonella typhimurium, Escherichia coli*, *Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus, respectively* at 5 mg/mL of CuO nanoparticles, the same process was repeated with the ZnO NPs synthesized using the same bacteria which revealed that CuO NPs exhibited better inhibitory effects against all bacteria in comparison of ZnO NPs probably due to their interaction with bacterial protein via the SH groups thus inactivating the growth of bacteria (Figure 19c) (Mohamed et al. 2021).

The textile wastewater has a high percentage of contaminants such as hardness, high turbidity, pH, TSS (total suspended solids), TDS (total dissolved solids), COD (chemical oxygen demand), sulphates, chlorides, and many other impurities which causes the death to aquatic organisms. The treatment with biogenic Cu NPs obtained using *Escherichia sp.* by Noman *et al*. (Noman et al. 2020) revealed their potential to decolorize the azo dyes (25 mg L^{-1}) up to 83.61% ± 1.93, 88.42% ± 2.80, 90.55% ± 2.06 and 97.07% ± 1.22, and for RB-5, DB-1, MG, and CR in 5 hours of sunlight exposure (Figure 18d). *Streptomyces sp.* (Endophytic actinomycetes) mediated CuO NPs showed their potential in biotechnological applications (Hassan et al. 2019). *Aspergillus niger* strain STA9 assisted Cu NPs displayed antibacterial, antidiabetic, and anticancer activities (Noor et al. 2020).

5.6 TiO_2 nanoparticles (TiO_2 NPs)

TiO_2 nanoparticles have been mostly studied and used for their brilliant antioxidant nature and superior photocatalytic properties. They are frequently used in implant biomaterials, photocatalysis, sunscreen products, toothpaste, self-cleaning sanitary ceramics, cement, sugar, paper, rubber, biomedical ceramic, printing ink, paints, antimicrobial plastic

packaging, films, etc. (Khataee and Mansoori 2011). Green synthesis of TiO_2 NPs by microorganisms has been studied and summarized in this section.

5.6.1 Synthesis and properties

Synthesis of TiO_2 NPs using *Streptomyces sp.* HC_1 was studied by Ağçeli and coworkers when they cultured the bacterial colony in sterile nutrient broth (50 mL) and incubated it for 24 hours in a shaker with 150 rpm at 37°C. This bacterial culture was used after the appearance of turbidity and added to $TiO(OH)_2$ solution (20 mL, 0.025 M), followed by incubation in a steam bath for 30 minutes at 60°C. After incubation, white clusters were noticed at the bottom of the flask which was collected by centrifugation, and the precipitate was washed with distilled water to maintain neutral pH (Ağçeli et al. 2020). *Bacillus sp.* bacteria was used for the synthesis of 50 nm-sized spherical shapes TiO_2 NPs. The synthesis of these bacteria-mediated nanoparticles was performed using the Taguchi method (which increases the reliability of production by optimizing the process parameters) and obtained nanoparticles revealed their highly dense spherical shape which was confirmed by TEM and SEM micrographs. The TGA studies suggested the weight loss up to 670°C in which the evaporation of water was responsible for first weight loss at below 150°C temperature while in second weight loss, TiO_2 nanoparticles and organic compounds were decomposed in the range of 250 to 670°C (Figure 20) (Moradpoor et al. 2021).

5.5.2 Applications

TiO_2 NPs exhibit outstanding contributions in photoelectrochemical energy production, and their biocompatible and non-toxic properties, render them a suitable candidate for biomedical applications and pharmaceutical industries. (Zhao et al. 2007; Weir et al. 2012; Ahn et al. 2018) The TiO_2 NPs synthesized using *Aspergillus flavus* showed their effect on the bacterial sp, *K. pneumoniae* (18 mm), *B. subtilis* (22 mm), *S. aureus* (25 mm), *P. aeruginosa* (27 mm), and *E. coli* (35 mm); for the control, tetracycline antibiotics was used and for the zone of inhibition, they deployed the cell diffusion method and MIC. The zone of inhibition was shown against both Gram-positive and Gram-negative bacteria. The MIC values were found to be 40 µg mL^{-1} for *E. coli* (MTCC-1721), 40 µg mL^{-1} for *S. aureus* (MTCC-3160), 45 µg mL^{-1} for *B. subtilis* (MTCC-1427), 70 µg mL^{-1} for *K. pneumoniae* (MTCC-4030), and 80 µg mL^{-1} for *P. aeruginosa* (MTCC-1034) suggested best results against *E. coli*, Figure 21 (Rajakumar et al. 2012) TiO_2 has also unveiled antibacterial activity against *Bacillus megaterium* (Karunakaran et al. 2013) and *E. Coli.* (Amin et al. 2009; Hong et al. 2017) using environmental light.

5.7 ZnO nanoparticles (ZnO NPs)

ZnO nanoparticles have been widely used as antibacterial, anti-fungal with some other biomedical applications, and as active photocatalysts for the degradation of dyes and other organic contaminants (Ong et al. 2018).

5.6.1 Synthesis and properties

Barani *et al.* (Barani et al. 2021) used two bacterial strains (*Vibrio* sp. VLA strains and Marinobacter sp. 2C8) for the synthesis of ZnO NPs. The ensuing precipitate after bacterial exposure was separated by centrifuged, washed, and dried by a freeze dryer. The peak in the UV was found at 250 nm ZnO-VLA while 266 nm for ZnO-2C8 nanoparticles. The shape of nanoparticles was found spherical within the range of 10.23 ± 2.48 nm size. The change in color to golden brown with some precipitate indicated the formation of nanoparticles which was centrifuged for 10 minutes at 10,000 g to separate the precipitate; their formation was determined by UV Visible spectroscopy which suggested the absorption maxima at 360 nm (characteristic peak for ZnO) and quantum size effect was analyzed with a blue shift

which is responsible for the diminution of size and wavelength due to widening of the bandgap. The 34.98 nm-sized and spherical-shaped nanoparticles were affirmed by TEM analysis while SAED analysis suggested crystals of 7 nm average size (Rafeeq et al. 2021).

Periconium sp. was used for ZnO NPs synthesis by applying the sol-gel process via dissolution of Zn $(NO_3)_2$ (20 g) in deionized water (100 mL) at 90°C with constant stirring. The addition of fungal extract (25 mL) was done at this stage and could form a sol by evaporation while the pH was maintained at 5. The sol was kept for more than 24 hours in a hot air oven at 45°C for evaporation of water resulting in the gel formation and even dispersal of Zn^{2+} ions. After drying for 12 more hours at 125°C, the color of gel changed to brittle yellow and porous ZnO NPs ensued after calcination for 4 hours at 700°C under the aerobic condition in a muffle furnace. The process of synthesis and morphology (studied by TEM micrographs) of ZnO NPs are depicted in Figure 22 (i) and (ii) (a-e), suggesting quasi-spherical (size~16 and 78 nm), polydisperse (polydispersity index ~ 1.48), and less agglomerated. The SAED patterns revealed a circular peripheral layer related to the planes suggested by XRD patterns (Ganesan et al. 2020).

In another process, a freshly grown cell-free supernatant of *Bacillus megaterium* was used with 1mM aqueous $ZnNO_3.5H_2O$ and kept on a shaker incubator for 48 hours at 37° C. The white clusters were obtained at the bottom of the flask after 12-48 hours of incubation which was centrifuged and washed several times to obtain pure white crystals of ZnO NPs; characteristic surface plasmon resonance peak at 346 nm in the UV-Vis spectrum confirmed their synthesis. XRD pattern supported the crystalline nature of synthesized nanoparticles with sizes in between 45 and 95 nm possessing cubic shape (Figure 23) (Saravanan et al. 2018).

5.6.2 Applications

The antioxidant activity of ZnO NPs synthesized via *Marinobacter sp.*2C8 and *Vibrio sp*. bacteria was evaluated by DPPH scavenging radicals. When the ZnO NPs react with DPPH radicals, pale yellow color ensues from the deep purple which showed the existence of 1,1-diphenyl-2-picrylhydrazine as a result of receiving electrons. The two sets, ZnO-2C8 and ZnO VLA, of nanoparticles, showed different activity from 31.2 µg/mL to 2500 µg/mL concentration; at increasing concentration of ZnO concentration, the DPPH radical scavenging activity percent also increased, suggesting a dose-dependent antioxidant activity. The maximum antioxidant activity was observed at 2500 µg/mL was 89% for ZnO-2C8 NPs and 86% for ZnO-VLA NPs; EC_{50} values for both being at 600 µg/mL, Figure 24 (Barani et al. 2021).

ZnO NPs fabricated from cyanobacterium *Nostoc* sp. EA03 showed its potential in biological functions in terms of antibacterial, antimicrobial, and toxicity activities. With these nanoparticles, MBC and IC_{50} values were determined to be 2500, 2500, and 128 µg mL^{-1}, and 2000, 2000, and 64 µg mL^{-1}, respectively which bodes well for their biomedical appliances (Khatami et al. 2018; Ebadi et al. 2019).

5.8 Fe_2O_3 nanoparticles (Fe_2O_3 NPs)

Iron oxide nanoparticles (IONP)predominantly exist in two forms which are magnetite (Fe_3O_4) and the oxidized form called maghemite (γ-Fe_2O_3) (Markova et al. 2014; Plachtová et al. 2018). The Fe_2O_3 NPs attracted much attention due to their unique properties of super magnetism, and their appliances in various areas including terabit magnetic storage, catalysis, gene, and drug delivery, and other therapeutic applications (Can et al. 2012). When the high surface energy possessing Fe_2O_3 NPs react with the biomolecules, it results in the enhancement of dispersion and less aggregation due to the presence of polysaccharides which offer a brilliant biocompatible shell (Ghosh et al. 2021).

5.8.1 Synthesis and properties

For the synthesis of Fe_2O_3 NPs by three strains of fungus, i.e., *Fusarium incarnatum, Phialemoniopsis ocularis*, and *Trichoderma asperellum*, initially, fungal cell filtrate (10 mL) of fungal strains were mixed with the salt solution of $FeCl_3$ and $FeCl_2$ (2:1 mM). The mixture was allowed to agitate at room temperature for 5 minutes when the change in color of the reaction mixture, indicated the formation of nanoparticles with selected fungal strain. Later, the synthesized Fe_2O_3 NPs were centrifuged for 20 minutes at 12,000 rpm and washed thoroughly with deionized water; the entire process was described by Mahanty *et al.*(Mahanty et al. 2019) in the flowchart (Figure 25). Aqueous extract of *Aegle marmelos* (5g) was used for the synthesis of Fe_2O_3 NPs with 100 mL distilled water and stirred with heating for 1 hour followed by the filtration of the extract using Whatman filter paper. 10 mL of this extract was mixed with 90 mL of ferric nitrate and stirred for 1 hour and then kept in a hot air oven while the temperature was maintained at 150°C. Later, the powder was calcinated for 5 hours at 400°C to generate Fe_2O_3 NPs (Sriramulu et al. 2021).

5.8.2 Applications

The Fe_2O_3 NPs synthesized using *Aegle marmelos* extracts inhibited the bacterial growth of *E. coli and S. aureus* more than the control antibiotic. The gram-negative bacteria (*Escherichia coli*) interacted more with nanoparticles at a higher and lower concentration as compared to gram-positive bacteria (*Staphylococcus aureus)* due to the difference in cell membrane thickness. The nanoparticles kill the bacterial cell by entering the membrane and inhibiting the bacterial growth and inactivated the enzymes with the increase of the cytoplasmic membrane leakage (Figure 26a); nanoparticles at concentration 32.25 µg/ mL showed 7 ± 0.12 mm inhibition for *E. coli*.(Khalil et al. 2017) Against *S. aureus*, 30 ± 0.387 mm and 28 ± 0.654 mm (30 µg/ mL) zone inhibition was observed while 21 ± 0.432 and 19 ± 0.547 (15 µg/ mL) was seen for *E. coli*, suggesting more inhibition of *E. coli* (Figure 26b). These Fe_2O_3 NPs exhibited superb photocatalytic activity for the degradation of BG dye with 95.89% degradation in 90 minutes under UV light; degradation efficiency and degradation kinetics suggested the pseudo-first-order kinetic model with the constant (K) value of 0.04058 min^{-1} (Figure 26 (c-e) (Sriramulu et al. 2021).

5.9 Se nanoparticles (Se NPs)

Se nanoparticles are attractive due to their low toxicity, good biocompatibility, excellent biological activities, and being essential for mammalian's life; as a trace element, they exhibit preventive properties for disease and exhibit good antitumor activity (Sun et al. 2014). It plays a key role in fighting against oxidative stress by participating in the antioxidant defense system of the liver.(Kondaparthi et al. 2019) On the other hand, selenium sulfide is an antifungal medicine and bioactive chemical. However, its biosynthesis is always an issue of controversy when one discusses nanoparticle forms.

5.9.1 Synthesis and properties

Hashem *et al.* (Hashem et al. 2021) synthesized Se NPs using *Bacillus megaterium* bacteria wherein the bacteria were cultured and incubated at 37°C for 48 h with shaking aerobically followed by the removal of bacterial cells and macromolecules by filtration through 0.44 µm PVDF filter and centrifugation at 10,000 rpm. Later, selenious acid suspension (1 mM) was mixed with cell-free supernatant and stirred at 25°C. The synthesis of Se NPs by reduction was indicated by a color change from colorless to reddish color when they were centrifuged for more than 30 minutes at 12,000 rpm. DLS and TEM studies suggested the synthesis of 41.2 nm-sized monodispersed spheres. The fungus, *Saccharomyces cerevisiae* was used to fabricate the SeS nanoparticles via the addition of selenium salts in 1 mM concentration into the 24 hours culture of *Saccharomyces cerevisiae* and incubation for 4.5 hours at 35°C with 180 rpm shaking using a shaking incubator Next, the medium changed the color from yellow to brownish red for

S_1(synthesized using sodium selenosulfide salt) in 18 hours while for S_2 (using selenous acid/sodium sulfite) in 4.5 hours. The presence of nanoparticles was confirmed by optical microscopy and these results were supported by TEM and SEM analysis which revealed 6.0 size for S_1 and 153 nm size for S_2 nanoparticles. The characteristic peaks of SeS nanoparticles were confirmed by XRD spectra as well as mass spectroscopy. The process of SeS NPs fabrication has been given in Figure 27 (Asghari-Paskiabi et al. 2019).

Afzal *et al.* (Afzal et al. 2021) used cyanobacteria for Se NPs synthesis as affirmed by the synthesis of spherical and amorphous nanoparticles of 10.8 nm size. Freshly, *Stenotrophomonas bentonitica* BII-R7 bacterial strain was used to reduce Se(IV) to Se(0) NPs by biotransformation of amorphous nanospheres of Se(IV) to trigonal Se (0) NPs (Pinel-Cabello et al. 2021).

5.9.2 Applications

Sirsat *et al.* (Shirsat et al. 2015) reviewed the microbial-assisted synthesis of Se NPs and their applications with their appliances in various diverse fields such as medicine, sensors, electronics, energy, and space industries; assorted therapeutic applications of Se NPs are depicted in Figure 28.

These nanoparticles could inhibit the growth of *Alternaria, Candida, Aspergillus*, and the dermatophytes genera pathogenic fungi and MTT assay revealed their non-toxic nature (Asghari-Paskiabi et al. 2019). Se NPs produced using cyanobacteria showed biocompatibility and antioxidant, antimicrobial, anticancer activity when compared to chemically synthesized or commercially available Se NPs. The antioxidant activity was performed against ABTS, FRAP, DPPH, SOR assays, and ascorbic acid was used as the positive control. IC_{50} values for ascorbic acid, B-SeNPs (biogenically synthesized), and C-SeNPs (chemically synthesized) were found 84.71 ± 0.68, 92.58 ± 1.28 and 239.11 ± 0.34 µg/ mL in ABTS assays while 59.53 ± 0.53, 155.02 ± 0.93 and 178.89 ± 1.84 µg/ mL respectively found in FRAP assay. In DPPH assays, IC_{50} values for ascorbic acid, B-SeNPs, and C-SeNPs were 56.36 ± 1.52, 83.89 ± 2.11 and 174.79 ± 0.29 µg/ mL, respectively and for SOR assay, these values were 74.95 ± 0.95, 80.55 ± 1.14 and 176.84 ± 0.12 µg/ mL for ascorbic acid, B-SeNPs, and C-SeNPs, respectively, Figure 29 (Afzal et al. 2021).

Lactobacillus casei ATCC 393 assisted prepared Se NPs inhibited the colon cancer cell growth which was studied in BALB/c mice's CT26 syngeneic colorectal cancer model. The nanoparticles showed *in vitro* antiproliferative activity, induced apoptosis, and raised ROS levels in cancer cells (Spyridopoulou et al. 2021).

6. Advantages And Disadvantages Of The Nanoparticle's Synthesis Using Microorganisms

Several microorganisms have been used for the sustainable synthesis of nanoparticles such as metals, quantum dots, semiconductors, etc. having different sizes and shapes (Narayanan and Sakthivel 2010). In comparison to conventional synthesis, green synthesis is cheaper, eco-friendly, and non-toxic (Mahmoud 2020). Adopting the microbial route has numerous advantages such as the microbes having a high growth rate and being inexpensive to cultivate (Prasad et al. 2016). They are easy to handle and can be genetically manipulated or modified without much difficulty (Bhattacharya and Gupta 2005). The process for the synthesis of nanoparticles using microbes is very simple, stable, and robust that leading to higher production rates (Nikolaidis 2020). In addition, the nanoparticles synthesized using microbes revealed high surface area, as well as these, were monodispersed (Singaravelu et al. 2007).

Although the microbial synthesis route showed several advantages yet some disadvantages needed to be noticed such as low repeatability and the process for getting the clear filtrates from colloidal broths involving the use of

sophisticated equipment. However, genetic manipulation is in demand but for the fungal platform is still challenging (Grasso et al. 2020). Moreover, pure nanoparticles are hard to obtain that are lack biomolecules and capping agents. Also, there is a need for thorough research for large-scale production and applications.(Prasad et al. 2016)

7. Conclusion And Future Perspective

In recent years, sustainable or green synthesis has received considerable attention due to its economic importance as it provides a clean, facile, effectual, non-toxic, and eco-friendly route for the synthesis of nanoparticles. Microbes-assisted metal or metal oxide nanoparticles with extremely ordered structures demonstrated their potential for numerous applications in waste treatment, biological and therapeutic fields due to their biocompatibility, controlled morphology, and other useful endowed properties in nano form. Some unique features of microbial cells that promote their use in the synthesis of nanoparticles as reducing and capping agents comprise easy maintenance, fast growth, and safer use. The greener synthesis addresses the bottlenecks for the synthetic methods due to the coating of nanoparticles with the biomolecule or lipid layer which endows physiological solubility, processibility, and stability to the nanoparticles as it enables surface functionalization for applications in biomedical fields.

However, the nanoparticles synthesized adopting the green route face some challenges which need to be addressed namely the slow rates of synthesis and stability of nanoparticles. The problems can be circumvented by augmenting the methods of cultivation of microbes and techniques of extraction and optimization via numerous combinatorial approaches, for example, photobiological methods. The other challenge is the production rate which is quite low in the case of biosynthesis (1/3 in comparison of chemical synthesis) which needs to be surmounted for their applications in real-world systems for large-scale applications. In addition, the lack of monodispersity, variations from batch to batch, and time-consuming process also limit their use in the commercial world. All the underlying mechanisms for the synthesis of nanoparticles including biochemical, cellular, and molecular mechanisms should be researched in detail for enhancing the properties, rate of synthesis, and applications of these nanoparticles. Biological protocols should be kept in mind before the synthesis of such nanoparticles namely the type and inheritable properties of organisms, ideal conditions for enzyme activity and cell growth as well as the reaction conditions. Although researchers are focusing on the therapeutic effect of nanoparticles yet the other important aspect is their toxic side effects. In the absence of biodegradation, delayed elimination trailed by the intercellular reactive oxygen species generation, damage to DNA, apoptotic cell death, and long-term toxicity can be caused by nanoparticles. Till now, most of the microbes-assisted nanoparticles have been examined in *in-vitro* studies for biomedical use and clinical trials on large scale to assess their safety is an important aspect for their effects in *in-vivo*. Hence, some factors such as reduction or removal of toxicity, doses, and response to host immune system throughout treatment are some aspects that still need to be addressed before the commercialization of these nanoparticles. The “green chemistry” concept combined with the “white biotechnology” approach can lead to a major achievement in many sustainable industrial developments with the use of genetically modified organisms by understanding the mechanistic aspects and related metabolic pathway culminating in the enhancement of efficiency for the synthesis of nanoparticles with reduced toxicity (Iravani and Varma 2019). Hopefully, with further thorough investigations, the microbes-assisted nanoparticles will attain their immense potential in various sectors of nanotechnology using genetically engineered organisms.

Abbreviations

4-AP - 4-amino phenol

BET - Brunauer-Emmett-Teller

BG - Brilliant green

CLSI - Clinical and Laboratory Standards Institute

CR - Congo red

CuO - Copper oxide

Cu_2O - Cuprous oxide

DLS - Dynamic light scattering

2,4- DNPH – 2,4-dinitrophenilhydrazine

EDS - Energy dispersive spectroscopy

FESEM - Field emission scanning electron microscopy

$FeCl_3$ - Ferric trichloride

$FeCl_2$ - Ferric dichloride

FTIR - Fourier transform infrared

GO - Graphene oxide

HAuCl - Chloroauric acid

MBC - Minimum bactericidal concentration

MICs - Minimum inhibitory concentrations

MLCs - Minimum lethal concentrations

MB - Methylene blue

MO - Methyl orange

4-NP - 4-nitrophenol

SAED - Selected area electron diffraction

SERS - Surface-enhanced Raman scattering

$ZnSO_4.H_2O$ -Zinc sulfate monohydrate

Declarations

Conflict of interest:

The authors declare no conflict of interest.

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Figures

Figure 1

Assorted methods for the synthesis of nanoparticles (reproduced from Ref. (Jadoun et al. 2021) with permission)

Figure 2

The number of articles published on green synthesis of nanoparticles using algae, fungi, bacteria, yeast, and other microorganisms over the last decades (Data from the ISI Web of Knowledge database).

Figure 3

Flowchart of microbial synthesis of nanoparticles and their applications.

Figure 4

Schematic representation of scheme of green synthesis of nanoparticles (nanorods, nanowires, nanoconjugates, and nanotubes) via microbes (bacteria, fungi, algae, yeast, and virus) (Reproduced from Ref. (Gahlawat and Choudhury 2019) with permission)

Figure 5

Schematic representation of the intracellular synthesis of nanoparticles by microorganisms. (NP – nanoparticles, N – nucleus, +ve = ions, −ve = charges on the cell wall

Figure 6

Green synthesis of Ag/AgCl nanoparticles derived from *Chara vulgaris* algae extract and its antibacterial applications (Reproduced from Ref. (Hassan et al. 2021a) with permission)

Figure 7

Microwave-assisted green synthesis of Ag NPs using crude extracts of *Boletus edulis* and *Coriolus versicolor*: Characterization, anticancer, antimicrobial and wound healing activities (Kaplan et al. 2021)

Figure 8

Proposed scheme for green synthesis of silver (Ag) nanoparticles using yeast extract and antibacterial activity. (Reproduced from Ref.(Shu et al. 2020) with permission)

Figure 9

The schematic diagram for the synthesis of silver (Ag) by using (a) Bacteria (b) Fungi (c) Algae (Reproduced from Ref. (Rafique et al. 2017) and (Arya et al. 2019) with permission)

Figure 10

Green synthesis of Ag NPs using *Enterobacter sp.* (i) Observation of supernatant and UV-Visible spectra for the synthesis of Ag NPs (a) control supernatant of *Enterobacter sp.* (SMP1) (a1) Control 1.5 mM $AgNO_3$ solution (b) Development of brown color after the addition of supernatant to $AgNO_3$ solution indicated the formation of nanoparticles (c) the appearance of Ag NPs peak at 450 nm in UV-vis absorption spectrum is a characteristic peak of Ag NPs assigned to surface Plasmon resonance of the particles. (ii) (a1 and b) Spherical morphology of Ag NPs by

TEM images. X-ray elemental mapping of (a2) Ag (red), (a3) nitrogen (blue), (a4) carbon (green), (a5) oxygen (yellow), and (a6) sulfur (orange) in which the area covered by Ag NPs is shown with the pink dotted line and the presence of characteristic elements are shown by intense color for respective elements detected (c) the crystalline nature of Ag NPs is shown by X-ray diffraction pattern and (d) the presence of silver is shown by energy dispersive spectroscopy (EDS) spectrum (Reproduced from Ref. (Ashraf et al. 2020) with permission)

Figure 11

Applications of Ag NPs in different fields (a) catalytic behavior of Ag NPs in the synthesis of benzimidazoles (b) antibacterial activity of Ag NPs against standard and multi-drug resistance strains. (Reproduced from Ref. (Arya et al. 2019) and (Nazari and Jookar Kashi 2021) with permission)

Figure 12

Representation for the synthesis of Au NPs from alga *Gelidiella acerosa* and its alpha-glucosidase enzyme inhibition property (Reproduced from Ref. (Senthilkumar et al. 2019) with permission)

Figure 13

Green synthesis of Au nanocatalyst for (a) high-efficiency degradation of quinclorac (b) decolorization of azo dye (Reproduced from Ref. (Shi et al. 2017) and (Qu et al. 2017b) with permission)

Figure 14

Antiproliferative activity of Au NPs against colon cancer cell lines. (A) Observation of cytotoxicity of Au NPs comparison with a standard (B) Morphological observation of HCA-7 cell line by the treatment of Au NPs (Reproduced from Ref.(Shunmugam et al. 2021) with permission).

Figure 15

Green synthesis of Pt NPs (a) using microorganisms as reducing and capping agent, the change in color and its spherical morphology (b) two-cycle two-electron mechanism for bioreduction Pt NPs (c) Size distribution of *Neurospora crassa* assisted Pt NPs (Reproduced from Ref. (Govender et al. 2009; Castro-Longoria et al. 2012; Puja and Kumar 2019) with permission)

Figure 16

Antioxidant activity of Pt NPs synthesized using various microbial strains (ADR19 - *Pseudomonas kunmingensis;* FZC6 - *Psychrobacter faecalis;* B-11177 - *Vibrio fischeri*; ZC15 - *Jeotgalicoccus coquinae*; KC19 - *Sporosarcina psychrophila* ; MN23 – *Kocuria rosea*; KT2440 - *Pseudomonas putida* ;CCV1 - *Rhodotorula mucilaginosa*) (Reproduced from Ref. (Eramabadi et al. 2020) with permission)

Figure 17

Green synthesis of Pd NPs using *Botryococcus braunii* algal extract (Reproduced from Ref. (Anju et al. 2020) with permission)

Figure 18

Biosynthesis of Cu NPs using *Escherichia sp.* (Reproduced from Ref. (Noman et al. 2020) with permission)

Figure 19

Anti-biofilm activity of the CuO NPs against Gram-positive and Gram-negative strains. (a) against *Staphylococcus aureus* (b) against *Pseudomonas aeruginosa* (c) Antibacterial activity for CuO NPs against different pathogenic bacteria at (5 mg/mL) (d) wastewater treatment by Cu NPs synthesized using *Escherichia sp.* (Reproduced from Ref. (Noman et al. 2020) and (Mohamed et al. 2021) with permission)

Figure 20

Microbial synthesis of TiO_2 NPs using Bacillus sp. (Reproduced from Ref. (Moradpoor et al. 2021) with permission)

Figure 21

Antimicrobial activity and zone of inhibition of TiO_2 NPs against (a) *Staphylococcus aureus*, (b) *Escherichia coli,* (c) *Pseudomonas aeruginosa*, (d) *Klebsiella pneumoniae*, and (e) *Bacillus subtilis. (*Reproduced from Ref.(Rajakumar et al. 2012) with permission)

Figure 22

Systematic diagram for the (i) Sol-gel synthesis of ZnO NPs using *Periconium sp.* extract (ii) TEM images (a–e) of the ZnO NPs attained at various magnification and (f) their respective SAED (selected area electron diffraction) pattern (reproduced from Ref. (Ganesan et al. 2020) with permission).

Figure 23

Green synthesis of anisotropic ZnO NPs using *Bacillus megaterium*, the characteristic peak appeared in UV and cubic shape particles by TEM (Reproduced from Ref. (Saravanan et al. 2018) with permission)

Figure 24

The antioxidant activity of biogenic ZnO NPs via *Marinobacter* sp. 2C8 and *Vibrio* sp at different concentrations where ascorbic acid was used as a standard (Reproduced from Ref. (Barani et al. 2021) with permission)

Figure 25

Flowchart for green synthesis of Fe_2O_3 NPs by fungi (Reproduced from Ref. (Mahanty et al. 2019) with permission)

Figure 26

Applications of Fe_2O_3NPs in antibacterial and dye degradation (a) mechanism of antibacterial activity of *Aegel marmelos* mediated iron oxide NPs (b) antibacterial activity of iron oxide NPs against *Escherichia coli* and *Staphylococcus aureus* (c) UV–Visible analysis of brilliant blue dye degradation with time (d) % of degradation (e) kinetics study of brilliant blue dye degradation using iron oxide NPs (Reproduced from Ref. (Sriramulu et al. 2021) with permission)

Figure 27

Pictorial representation of the green synthesis of SeS NPs by Saccharomyces cerevisiae along with its properties, antifungal activity, and cytotoxicity (Reproduced from Ref. (Asghari-Paskiabi et al. 2019) with permission)

Figure 28

The numerous therapeutic and other benefits of Se NPs

Figure 29

Comparative anti-oxidant assays of ascorbic acid, B-SeNPs (biologically synthesized Se NPs), and C-SeNPs (chemically synthesized Se NPs) (a) DPPH scavenging assay (b) SOR scavenging assay (c) ABTS assay (d) FRAP assay (Reproduced from Ref. (Afzal et al. 2021) with permission)