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Abstract

Modern life is not conceivable without the involvement of nanoparticles. Major aspects of science are dependent on nanotechnology for varied applications. The nanoscale size of nanoparticles, larger surface/volume ratio, characteristic structures, and similar dimensions to biomolecules led to their application in biomedical sciences. In the due course of development, nanoparticle synthesis has come a long way from conventional physical and chemical methods to latest method involving environment-friendly biological synthesis. Microorganisms like bacteria, fungi, blue-green algae, and yeast have been screened and adopted for biological synthesis of variety of metallic nanoparticles. This chapter discusses the detailed mechanism of microbe-mediated biosynthesis of nanoparticles, their applications in healthcare mainly as antimicrobials, and attempts at optimizing the factors influencing the quality of nanoparticles along with the newer dimensions and limitations of this arena.

Keywords

Nanoparticles · Green synthesis · Biogenic synthesis · Optimization

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29.1 Introduction

Nanotechnology is the branch of science and engineering that involves the design, production, and application of structure, and systems by manipulating atoms and molecules at the nanoscale, i.e. having at least one dimension in the order of 1 nm to 100 nm. It is concerned with the creation of nanoparticles (NPs) that exhibits unique chemical properties, controlled monodispersity, and more reactivity than parent metal atom owing to their high surface-to-volume ratio (Sahoo et al. 2021). NP consists of three layers: a surface layer equipped with a wide range of molecules, surfactants, metal ions, and polymers; a shell layer, above the core, which is the central portion of NP; and the NP itself (Shin et al. 2016). These are classified into different types based on their morphology, size, and shape. Organic NPs include ferritin, micelles, dendrimers, and liposome. Being biodegradable and non-toxic, they are widely used in drug delivery. Inorganic NPs are biocompatible, hydrophilic, and non-toxic besides being more stable than organic ones, and include both metallic as well as non-metallic oxide NPs. Ceramic NPs are non-metallic solids having wide applications in the photodegradation of dyes, photocatalysis, catalysis, and imaging. Bio-nanoparticles are an aggregate of atoms/ions/molecules, which are prepared in the living system naturally (Ijaz et al. 2020).

NPs have been reported to improve crop yield, seed germination, and total biomass of crops (Ali et al. 2021). Metal-based NPs, in particular, have gained significance in the agriculture sector as nanobiofertilizers and nanobiopesticides. Different types of metallic NPs have been created as they exhibit unique physical, chemical, and biological properties and have broad-spectrum applications in areas such as drug delivery, gene transfer, management of insect pests and agriculture and as antibacterial agents (Ghosh et al. 2008; Rai et al. 2011).

There have been innumerable attempts on researching and analyzing the most economical and efficient ways to synthesize the NPs. These are synthesized by physical methods such as the ball milling method, pulsed wire discharge method, and pulse laser ablation. Chemical production of NPs is an expensive, harmful, time-consuming, and low-yielding process; whereas, physical methods require sophisticated and high power consuming equipment (Khodashenas and Ghorbani 2014). Recently, the focus has shifted towards reducing the production cost and to enhance the properties of NPs mediated by various biological systems, which are eco-friendly and safer to use. It involves the production of NPs using a living source, bacteria, fungi, plant extract, etc., and is termed a biological or 'Green' synthesis (Mukunthan and Balaji 2012). This chapter describes the biogenic synthesis of NPs via microorganisms, related aspects such as the mechanism taken up by them to reduce metal ions into its nano-form, and factors influencing the same. In addition, it cites recent developments happening in this particular field along with future prospects.

29.2 'Green' Synthesis of NPs

Conventional physical and chemical methods of synthesizing the NPs are associated with their respective limitations. Physically synthesized NPs are formed by a top-down approach in which bulk material is broken down into nano-size particles. Although it is a fast manufacturing process, a high requirement of energy makes it unsuitable for large-scale production. It also leads to imperfection in surface structure, which affects other properties of the material (Kandasamy and Prema 2015). Chemical methods viz., chemical reduction, microemulsion, and solvothermal decomposition, employ a bottom-up approach using organic or inorganic chemicals acting as reducing (sodium citrate, sodium borohydride, and sodium hydroxide) and stabilizing/capping (PVA, PVP, polyethylene glycol, and polymethacrylic acid) agents (Satyanarayana and Reddy 2018). In the chemical and physical method of NPs synthesis, highly toxic, expensive, and hazardous chemicals are used which are harmful to the environment. The biological method is preferred, as it does not involve toxic chemicals for the synthesis of NPs. It is a non-toxic and energy-efficient method. Herein, NPs are formed by the bottom-up/constructive method by reducing and stabilizing metabolites present in the microbial supernatant/microbial cell or plant extract; thereby, there is no requirement of adding these agents separately (Salem and Fouda 2021). In addition to this, the biogenic method is environmentally safe, and is being seen as a more sustainable way of NPs production as compared to the other conventional methods in use.

29.3 Microbe-Mediated Biogenic Synthesis of NPs and Their Applications

Production of NPs through microorganism is considered one of the most efficient ways of synthesizing NPs, as they are less toxic and covers a wider area of applications. Different types of microbes utilize different kinds of mechanisms to synthesize NPs (Rani et al. 2022). Reducing enzymes, mainly reductase, and stabilizing or capping agents present in microbes help in the conversion of metal into its nanoscaled particles (Rao et al. 2017). Stabilizing and capping agents prevent aggregation, therefore, lead to the formation of stable NPs. On the basis of the location of NPs formation, it can be differentiated as intracellular and extracellular synthesis. The reduction of metal ions into their nanoform within the cell is referred to as the intracellular mode of synthesis. It starts by binding oppositely charged metal ions to the carboxylate groups of the metabolites like polypeptides, and enzymes, present on the microbial cell wall (Zhang et al. 2011). This binding is necessary to facilitate the trapping of metal ions inside the cell, which are reduced to their atomic form by the reducing agents such as NADH-dependent reductase, present in the microbial cell. Then, these atomic nuclei undergo growth leading to the formation of aggregates and ultimately NPs after being stabilized by amino acids such as tryptophan, cysteine, tyrosine, and proteins/peptides (Iravani 2014; Shedbalkar et al. 2014). NPs are found to accumulate in the cytoplasm as well as

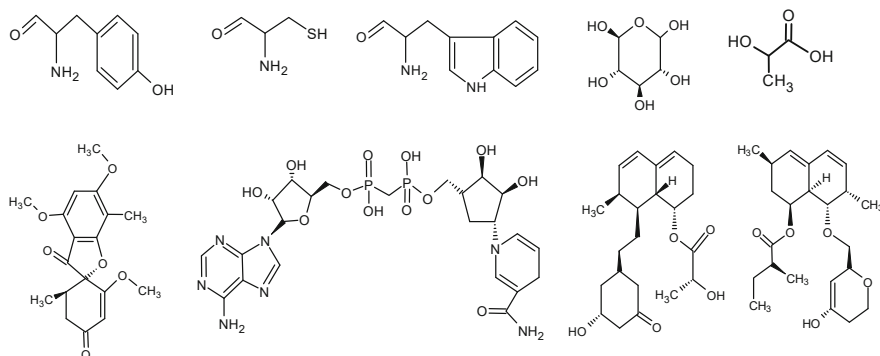


Fig. 29.1 Structures of the chemical compounds involved in the synthesis of nanoparticles from microbes (tyrosine, cysteine, tryptophan, glucose, lactic acid, griseofulvin, NADPH, mevastatin, and lovastatin)

cell wall (Mukherjee et al. 2001; Yusof et al. 2019). The extracellular mode of synthesis is facilitated by the extracellular enzymes that are either located on the cell membrane or released into the growth medium and mediate the reduction of metals from their ionic to atomic form followed by nucleation to form NPs. A few extracellular proteins also acts as capping agents and stabilize the NPs thus formed (Yusof et al. 2019). A number of microbial metabolites have been found to involve in the extracellular production of NPs (Ovais et al. 2018a, b; Ghosh et al. 2022). Barabadi et al. (2017) and Qamar and Ahmad (2021) discussed a few compounds that are involved in the synthesis of NPs from bacteria and fungi. Chemical structures of some of them have been depicted in Fig. 29.1.

There have been a number of attempts at synthesizing the metallic NPs from the microorganisms in recent times, some of which have been discussed below. Copper NPs are well known for their anti-microbial, anti-oxidant, and catalytic activity (Reddy Mallem 2022). Shantkriti and Rani (2014) reported the synthesis of CuNPs by incubating cell-free culture supernatant of non-pathogenic bacteria *P. fluorescens* with CuSO_4 (Copper sulfate) solution for 90 min. Lv et al. (2018) used *Shewanella loihica* PV-4 to produce CuNPs having antibacterial activity against *E. coli*. Noman et al. (2020) studied the production of CuNPs from the bacteria *Escherichia* sp. within a size range of 22.3 to 39 nm that acts as a photo catalyst during dye degradation. ZnONPs, widely used in antibacterial creams, lotions, ointments, mouthwashes, and paints, also act as antimicrobial agents and biofilm growth inhibitors (Mandal 2016). Spherical Zinc NPs within a size range of 14.39–37.85 nm were reported to be synthesized from the supernatant of a microalgae, *Cladophora glomerata* (Abdulwahid et al. 2019). A yeast, *Pichia kudriavzevii*, was also employed to prepare hexagonal zinc NPs by Moghaddam et al. (2017). Nadeem et al. (2018) reported bacterial strains, *Bacillus subtilis*, and *Lactobacillus* sp., as a source for the bio-production of TiO_2 NPs. Khan et al. (2021) reported that iron oxide magnetic nanoparticles synthesized by *Pseudomonas aeruginosa* are used in many applications, such as magnetic resonance imaging,

diagnostics, and therapeutics. Magnetic NPs with narrow-size distribution help in maintaining the temperature needed as per the exact calculations for cancer treatment. Iron NPs synthesized using *Proteus vulgaris* ATCC-29905 proved to be excellent anticancer and antimicrobial agents (Nadeem et al. 2021). Spherical AgCl (silver chloride) NPs with a centered cubic crystal structure and a mean particle size of around 10–50 nm synthesized by cell-free culture supernatants of *Pantoea agglomerans* and *Raoultella planticola* exhibited antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella*, and *Bacillus amyloliquefaciens* (Ghiuta et al. 2021). Alsamhary (2020) studied the antibacterial efficacy of *Bacillus subtilis*-mediated silver NPs to treat MDR (multidrug-resistant) microorganisms. Jebur and Abd (2021) synthesized MgO (magnesium oxide) NPs having different average diameters by two *Streptococcus* sp., *S. salivarius* and *S. mutans*. These NPs showed antibacterial efficacy against MDR, Gram-positive bacterium, *Acinetobacter baumannii* with a MIC (minimum inhibitory concentration) of 250 µg/mL. In a similar study, Fouda et al. (2021) investigated the antimicrobial potential of MgO-NPs (Source-*Penicillium chrysogenum*) which exhibited a zone of inhibitions at 200 µg/mL against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*.

29.4 Factors Affecting the Microbial Synthesis of NPs and Optimization Studies

Microbial species, the concentration of metallic precursor solution, incubation and reaction time, varying concentration of supernatant, pH, and temperature, are the factors that influence the quantity and quality of NPs significantly (Patra and Baek 2014). Not just the metabolites composition of each microbial species is different, but the pathways taken up to synthesize the NPs are also distinct. HR-TEM analysis revealed variations in the size and shape of NPs when the same microbe took up extracellular and intracellular modes of synthesis (Mohd Yusof et al. 2020). Different precursors of similar metals are used along with its varying concentration that influences the characteristics of NPs viz., shape and size. Therefore, a number of studies have been taken up to optimize these parameters in order to obtain NPs of desired quality and yield. One factor at a time (OFAT) is one of the most common approaches to carry this out that involves varying one factor at a time while keeping other factors as standard. Ebadi et al. (2019) optimized the synthesis of ZnONPs from the cell extract of cyanobacterium *Nostoc* sp. EA03. The maximum quantity of ZnONPs was obtained at a slightly alkaline pH (9) and 1000 µL cell extract concentration. Similarly, Bukhari et al. (2021) optimized the parameters that came out to be the concentration of Cu metal precursor solution (5 mM), reaction time (60 min.), filtrate to substrate ratio (1:1), and pH (7), from marine *Streptomyces* sp. Table 29.1 depicts various metallic NPs that have been optimized for different parameters affecting their biological synthesis from the microbes.

Table 29.1 Optimized parameters for the microbial synthesis of metallic NPs

NP	Source	Optimized conditions	Size (nm)	Applications	Reference
Ag	<i>Bacillus subtilis</i>	T (37 °C), pH (7.5)	2–26	Antibacterial properties against <i>Escherichia coli</i> , and <i>Staphylococcus aureus</i>	Yu et al. (2021)
	<i>Streptomyces</i> sp. SSUT88A	T (37 °C), pH (7), incubation time (5 days)	74.12	Antimicrobial activity against <i>Pseudomonas aeruginosa</i>	Rosyidah et al. (2021)
	<i>Chlorella vulgaris</i>	pH (12), incubation time (24 h), concentration of AgNO ₃ (3 mM), extract ratio (8:2)	55	Photocatalytic degradation of methylene blue	Rajkumar et al. (2021)
	<i>Aspergillus sydowii</i>	T (50 °C), pH (8.5), substrate concentration (1.5 mM)	1–24	Antifungal activity against clinical pathogens	Wang et al. (2021)
	<i>Cedecea</i> sp.	T (50 °C), incubation time (8 h), silver salt concentration (1.5 mM)	10–40	Antimicrobial activity against <i>Staphylococcus epidermidis</i> and <i>Staphylococcus aureus</i>	Singh et al. (2021)
	<i>Kocuria</i> sp.	T (55 °C), pH (9.8), incubation time (15 h), Silver nitrate concentration (1 mM)	46.73	Antibacterial potential against <i>Escherichia coli</i> , <i>Salmonella typhimurium</i>	Kumar et al. (2022)
Au	<i>Agaricus bisporus</i>	T (28 °C), pH (7), reaction time (72 h), concentration of gold chloride (1:9)	10–50	Antibacterial potential against human pathogens	Krishnamoorthi et al. (2021)
Se	<i>Lactobacillus paracasei</i> HM1	T (35 °C), pH (6), reaction time (32 h), agitation speed (160 rpm)	56.91	Antifungal activity against animal pathogenic fungi	El-Saadony et al. (2021)
MgO	<i>Aspergillus terreus</i>	T (35 °C), pH (8), concentration	8–38	Inhibiting the growth of pathogenic	Saied et al. (2021)

(continued)

Table 29.1 (continued)

NP	Source	Optimized conditions	Size (nm)	Applications	Reference
		of Mg (NO ₃) ₂ .6H ₂ O (3 mM)		microbes, tanning effluent treatment, and chromium ion removal	
TiO	<i>Synechocystis</i> NCCU-370	pH (7), temperature (30 °C), reaction time (12 h)	73.39	Antimicrobial activity against <i>Candida albicans</i>	Siddiqui et al. (2022)
FeO	<i>Alcaligenes</i> sp.	T (70 °C), pH (13), concentration of FeCl ₃ (2 mM), incubation time (2 h)	50–7	Degradation of malachite green	Sharma et al. (2022)
	<i>Purpureocillium lilaceinum</i> MW831030.1	pH (9), concentration of ferrous sulphate solution (3 mM), incubation time (3 days)	57.9	Dye removal (Safranin) and antibacterial activity against <i>Pseudomonas aeruginosa</i>	Hammed et al. (2022)

29.5 Bimetallic NPs: A New Dimension of Microbe-Based Nanotechnology

Bimetallic NPs, composed of two metals, are prepared by reducing the ions of two different metals simultaneously. These have been reported to improve the catalysts property of the original single metal that creates a new property (Ramsurn and Gupta 2013). The synergy existing between these metals is the major cause of their multifunctionality. They act as effective catalyzers in a variety of reactions owing to their large surface area. Bimetallic nanostructures comprise segregated and mixed nanostructures. Two different metals possess ordered and random arrangements of atoms, in the case of mixed structures like intermetallic and alloy nanoparticles, respectively. Segregated nanostructures, on the other hand, usually involve the formation of an initial structure of one metal type followed by the addition of the second metal. Thus, these are further classified as subcluster structures (separate distribution of two metals with a shared interface), core–shell structures (metal core is surrounded by a shell of second metal), multishell core–shell structures (shells possess alternative arrangement forming a shape like onion rings), and multiple core materials coated by a single shell (Ferrando et al. 2008; Srinoi et al. 2018).

Exhibiting higher activity, selectivity, and stability, they have wide applications in imaging and biomedical devices including nano-medicines (Erkey 2011; Loza et al. 2020). Bimetallic nanocomposites act as sensors and help in the early diagnosis of the disease. They have the ability to detect even 2–3 cancerous cells present in the body and are emerging as a bright option in the diagnostics field (Sharma et al. 2019). They also demonstrate greater antioxidant activity and dye removal efficacy used for wastewater treatment as compared to monometallic NPs (Riaz et al. 2022). Merugu et al. (2021) used the toddy palm extract to synthesize copper-zinc bimetallic NPs having anti-bacterial activity against *Alcaligenes faecalis*, *S. aureus*, *K. pneumoniae*, and *Clostridium perfringens*. Similarly, Riaz et al. (2022) took up the synthesis of monometallic as well as bimetallic/alloy NPs of zinc and copper from the aqueous extract of *Mirabilis Jalapa*. An equal volume of the plant extract was added to the aqueous solution of zinc chloride and copper sulfate (precursor salts of both metals) at varying concentrations. The continuous change in color from royal blue to sea green indicated the formation of nanoparticles. Fawzy et al. (2021) suspended cell pellet as well as cell-free supernatant of *Pseudomonas fluorescens* (PS1 and PS2) into ZnSO₄ and CuSO₄ solution wherein color change indicated the formation of bimetallic (Cu-Zn) NPs. Iron-manganese NPs synthesized using bacteria-fermented supernatant containing auxin complex compounds (indole-3-acetic acid) as reducing agents were evaluated as plant nanofertilizer. Spherical NPs showed a positive impact on seed germination, root development, and fresh weight of *Zea mays* (de França Bettencourt et al. 2020). They also act as promising antimicrobial agents besides displaying their tendencies in several industries affected by microbes such as water, food, textiles, and oil and gas (Arora et al. 2020).

29.6 Conclusion

Microbial synthesis of NPs is expected to take a leap forward in the near future owing to its environment friendliness, lesser cost of production, and biocompatibility provided it address a few limitations (Ovais et al. 2018a, b). A slower reduction process, reproducibility issues, and requirements of entirely aseptic infrastructure, besides unelucidated mechanisms underlying the same, can halt the synthesis as well as the application process (Rani et al. 2022). Therefore, besides focusing on exploring various microbial taxa for the synthesis of metallic NPs, researchers should also focus on the details of the reduction and stabilization process. In addition, the procedures involved in the extraction and purification of NPs from microbes needs to be studied in order to facilitate the full-fledged use of this application.

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