Journal of Agricultural Sciences (Tarim Bilimleri Dergisi) 2025, 31 (3) : 640 – 669 DOI: 10.15832/ankutbd.1622082

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Beneficial Microorganisms in Green Synthesis of Nanoparticles and Potential Applications

Gülden Kılıç[a]

[a]*Alanya University, Arts and Design Faculty, Gastronomy and Culinary Arts Department, Antalya, TÜRKİYE*

ARTICLE INFO
Review Article
Corresponding Author: Gülden Kılıç, E-mail: gulden.kilic@alanyauniversity.edu.tr, gulden-gk@hotmail.com
Received: 17 January 2025 / Revised: 21 March 2025 / Accepted: 22 April 2025 / Online: 29 July 2025

Cite this article
Kılıç G (2025). Beneficial Microorganisms in Green Synthesis of Nanoparticles and Potential Applications. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 31(3):640-669. DOI: 10.15832/ankutbd.1622082

ABSTRACT
Recently, green synthesis in nanotechnology has gotten considerable attention because of its economic importance, as well as providing a clean, eco-friendly, effectual, facile, and non-toxic route to nanoparticle (NP) synthesis. The utilization of various microorganisms especially beneficial microorganisms in NP synthesis presents a sustainable and eco-friendly alternative to conventional synthesis methods, aligning with the principles of green synthesis. In this regard, beneficial microorganisms used in fermented foods as starter cultures, such as *Lactobacillus acidophilus*, *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, *Secundilactobacillus kimchicus*, *Saccharomyces boulardii*, and *S. cerevisiae* have been utilized for the synthesis of Ag, Se, ZnO, Pd, Sb_2O_3, and TiO_2 NPs. These synthesized NPs have a high potential for use in drug delivery systems, agriculture, and the food industry as antimicrobial, antioxidant, and anticancer agents. Hence, further research is necessary on NP synthesis, novel sources for NP synthesis, and applications in various fields by considering its advantages and disadvantages. This review highlights the green synthesis of NPs, NPs synthesized by beneficial microorganisms, as well as the potential applications of NPs.

Keywords: Microbial synthesis, Fermented foods, Nanomaterials, Antimicrobial, Anticancer

1. Introduction

Nanotechnology is known as the reduction of atomic, molecular, and macromolecular matter to nanometer-scale molecules and is a highly prospective field of study in the twenty-first century, with applications spanning biotechnology, chemistry, medicine, and material sciences (Balakrishnan et al. 2017; Ahmed et al. 2024). Improving nanotechnology has synthesized various nanoparticles (NPs) and nanostructured materials. The particles with at least one dimension in 1-100 nm are described as NP, whose characteristics significantly change depending on the particle sizes (Pacheco-Blandino et al. 2012; Guleria et al. 2020). NPs feature inherent qualities that enable them to demonstrate reactivity and effectively bind, absorb, and transport a range of chemicals, such as DNA, RNA, small-molecule medications, proteins, and probes, with a significant level of efficiency (Elmer & White 2018). These NPs are very beneficial in various sectors such as materials and manufacturing, agriculture, food, medical, pharmaceutical, and environmental, as well as medical applications, ranging from diagnostics to cancer treatment (Guleria et al. 2020; Mitchell et al. 2021).

NPs consist of a wide variety of elements such as aluminum (Al), cerium (Ce), copper (Cu), gold (Au), iron (Fe), manganese (Mn), platinum (Pt), silver (Ag), titanium (Ti), thallium (TI) and zinc (Zn). Both bottom-up and top-down methodologies in NP synthesis are used (Singh et al. 2021). The bottom-up approach is a very effective method, wherein NPs occur by assembling smaller molecular components. In contrast, NP synthesis by the top-down approach involves utilizing a diverse range of techniques such as electro-explosion, milling, etching, lithographic processes, and laser ablation (Fu et al. 2018). These approaches are important in producing nanomaterials with excellent stability, and their morphology can change depending on desired applications (Jamkhande et al. 2019). Nonetheless, NPs produced can be highly toxic for humans and the environment, and high-cost equipment is also required for some physicochemical synthesis approaches, which increases their production cost and restricts their large-scale commercial production (Siaw et al. 2020). In this regard, the importance of green synthesis techniques has been increasing for the last decades. The major pathways of green synthesis techniques are schematized in Figure 1. The green synthesis of NPs has attracted great attention because of the biocompatibility, cost-effectiveness, low toxicity, and eco-friendly nature of the process and NP products (Mariotti et al. 2020). Also, there is a wide range of green synthesis methods for NPs, plant extracts, or the microorganisms are generally reacted with a metallic salt and then the biological reduction is performed to convert the metal to NPs. The obtained NPs are readily suitable for utilization after proper characterization (Mittal et al. 2013).

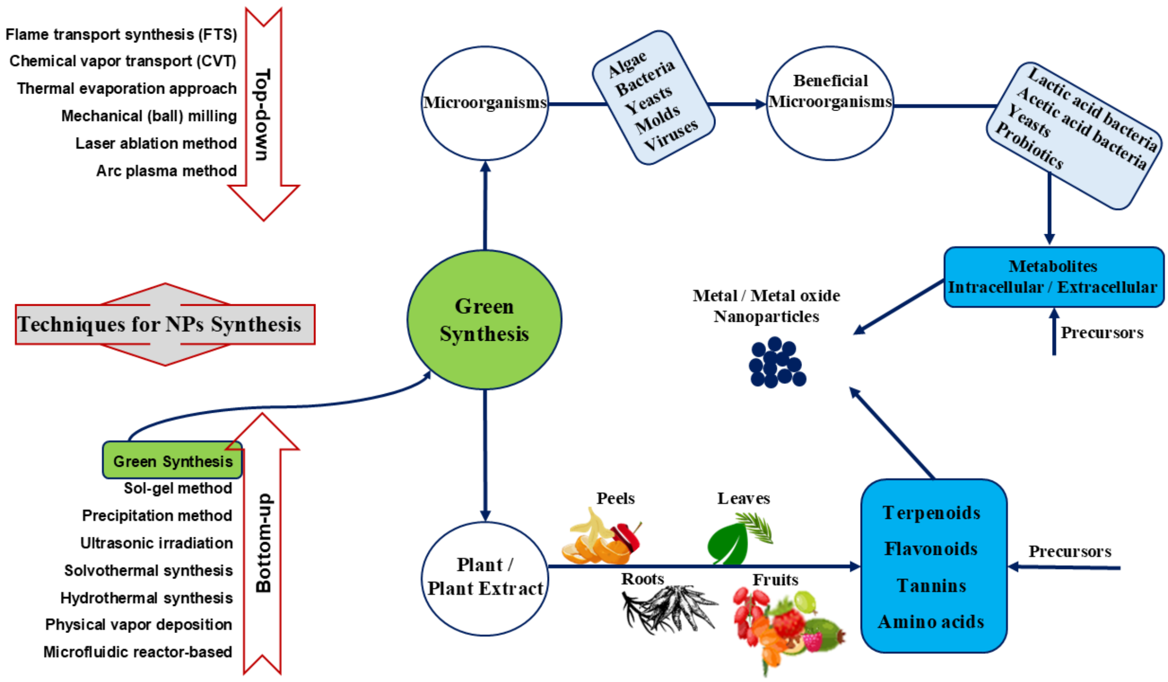

Figure 1- Schematic diagram for the green synthesis of nanoparticles

On the other hand, beneficial microorganisms, including probiotics, are frequently utilized in fermentations associated with foods/food applications and nutraceutical industries. These microorganisms with a positive effect on health are generally lactic acid bacteria (LAB), yeast, and acetic acid bacteria (AAB) (Hazards et al. 2017; Harandi et al. 2021; De Bellis & Rizzello 2024). The exceptional health benefits associated with AAB, LAB, and yeasts position them as highly promising biological scaffolds for the synthesis and targeted delivery of NPs. These microorganisms not only contribute to the sustainable and eco-friendly synthesis of NPs but also enhance their biocompatibility, stability, and therapeutic potential. By leveraging their unique metabolic pathways and bioactive properties, these microorganisms offer a versatile platform for optimizing NP formulations, improving drug delivery efficiency, and minimizing potential toxicity in various biomedical applications (Faramarzi et al. 2020; Majumder et al. 2022; Abd Qasim & Yaaqoob 2023; Mohammed et al. 2023). Various LAB and yeast species commonly found in foods have been used for NP (such as Ag and Zn) synthesis (Sásková et al. 2016; Abdulradha & Alhadrawi 2023). Generally, NPs produced by beneficial microorganisms are used for various purposes, such as antimicrobial and anticancer agents, in agriculture, drug delivery systems, and the food industry (Gomez-Zavaglia et al. 2022; Shanmugam et al. 2023). In this review article, the green synthesis of NPs, microbial synthesis, the usage of beneficial microorganisms for NP synthesis, and their potential applications are highlighted.

2. Green Synthesis of Nanoparticles

The present approach for NP synthesis is characterized by the absence of hazardous chemicals, elevated temperatures, high pressure, and providing that ensures both human health and environmental safety (Mariotti et al. 2020). The utilization of economically viable and ecologically sustainable substances, including botanical extracts, microorganisms (bacteria, fungi, microalgae, and viruses), organic polymers, and proteins, has solidified this methodology as a widely favored way for the synthesis of NPs (Taha 2022).

2.1. Nanoparticles from plants or plant extracts

Different parts of plants and vegetables, including peels, extracts, and shells, can be utilized in various applications. The active natural chemical compound obtained from these parts by removing their tissue with a solvent is a plant extract, which is also rich in terms of proteins, soluble fibers, bioflavonoids, and insoluble fibers (Doan et al. 2020; Priya et al. 2023). Plants have been accepted as a more reliable approach to the synthesis of nanomaterial (Figure 2) as they avoid the usage or toxic substances formation, are abundant and environment-friendly, and allow better control over NPs' morphology. Hence, the functional substances such as alkaloids, amino acids, carbonyl, ketones, phenolics, polysaccharides, proteins, vitamins, and tannins found in plants are used for NP synthesis as a reductant source by reducing metal ions into atoms (Vijayaraghavan & Ashokkumar 2017; Kumar & Rajeshkumar 2018). In this scope, NP synthesis has been utilized in plant extracts such as *Aloe vera*, *Camellia sinensis*, *Cinnamomum zeylanicum* leaf, *Jatropha curcas*, *Mangifera indica* leaf, *Mangosteen* leaf, *Murraya koenigii* leaf, gooseberry, mushroom, lemon, pear, papaya, and tansy. NPs prepared using the extracts ensure the advantage in long-term storage conditions due to the non-aggregation of NPs (Jayaprakash et al. 2017; Bhardwaj et al. 2020). Also, tea and coffee extracts were exploited to synthesize stable NP such as Pd and Ag NPs (Size ranges 20-60 nm). Accordingly, these approaches might be employed for NP synthesis of other noble metals (Nadagouda & Varma 2008).

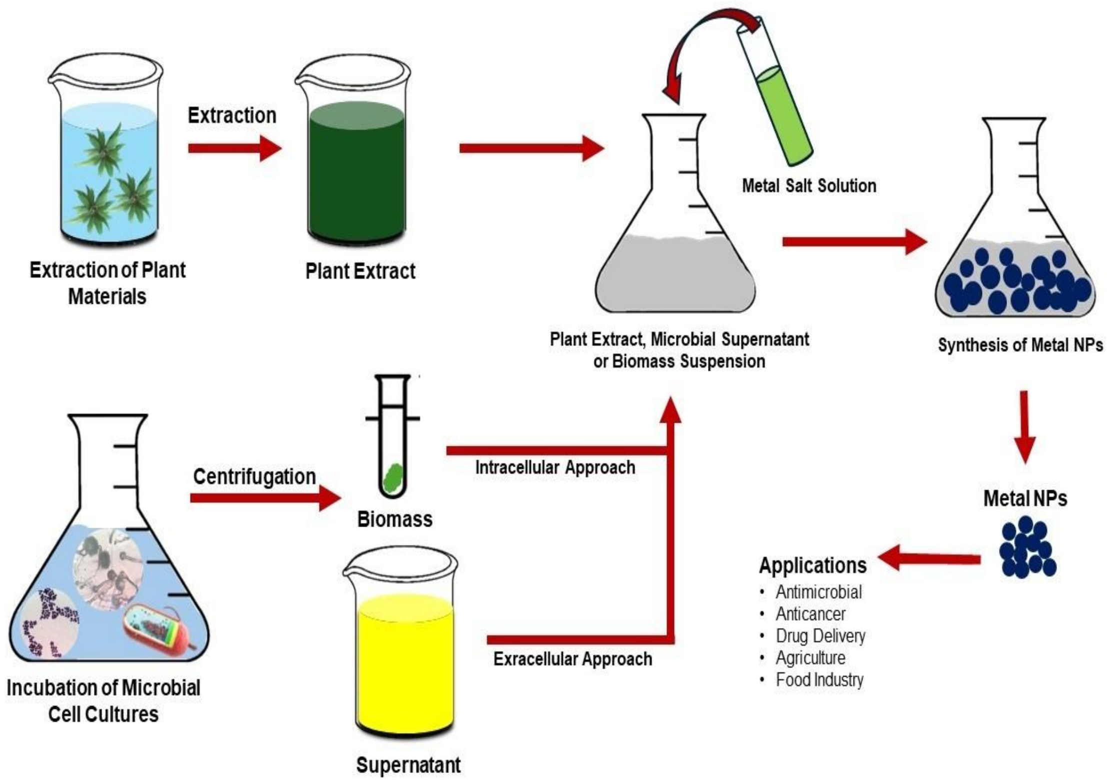

Figure 2- Schematic illustration for plant and microbial synthesis of nanoparticles

On the other hand, various plant sources were explored for the synthesis of metal NPs that had different characteristic properties. For example, in a study, spherical Ag NPs with sizes of 45-110 nm were synthesized using leaf extracts from Congolese plant species (*Brillantaisia patula*, *Crossopteryx febrifuga*, and *Senna siamea*). The study revealed that Ag NPs showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Kambale et al. 2020). In another study, Jayaprakash et al. (2017) produced Ag NPs from the natural fruit extract of *Tamarindus indica*. The plant extract acted as both a reducing and a capping mediator for Ag NPs synthesis. The morphology of NPs was a face-centered cubic shape and 6-8 nm sizes. Besides, they possess good antibacterial action. In a different study, Abbas et al. (2020) showed that plant-mediated NP synthesis could be used in wastewater treatment applications and for this purpose, Ag NPs and Au NPs obtained from *Aloe barbedensis*, *Azadirachta indica*, and *Coriandrum sativum* were fabricated. Table 1 compiles various plant sources for biogenic NP synthesis and their characteristics.

Plants provide advantages for natural product synthesis owing to their inherent safety, widespread availability, and diverse biomolecules or metabolites, which aid in stabilizing and reducing NPs (El-Seedi et al. 2019). Plant-derived nanotherapeutic medications have become a potential asset in contemporary cancer therapy. Over the past few years, substantial research has been dedicated to producing metallic NPs with potential anticancer properties. This exploration has spanned both laboratory experiments and studies conducted within living organisms (Andleeb et al. 2021). The anticancer properties of bioactive compounds obtained from various plants and/or plant extracts have been investigated (Majumder et al. 2019). It has been detected that the functional groups attached to NPs directly or indirectly improve anticancer effectiveness, reduce toxicity, and increase bioavailability/absorption (Pei et al. 2020). In a study conducted by In & Nieva (2015) examined the efficacy and toxicity of various NP-based chemotherapeutic treatments. It was detected that chemotherapy using NPs has substantially enhanced the management of non-small cell lung cancer compared to conventional chemotherapy. Also, Dutta et al. (2020) synthesized and stabilized orange peel-based NPs to induce apoptosis in hepatic cancer cell lines, while Andleeb et al. (2021) found that the effectiveness of various NPs in addressing cancer differs due to variations like the biological material used in their manufacturing process.

Table 1- Nanoparticle synthesis from various plant sources and their characteristics

Plant sources	*NP/Size/Shape*	*Characteristics*	*References*
Ananas comosus leaves	Ag NPs, 40-150 nm with hexagonal or spherical shape	Antimicrobial activity	Anis et al. 2023
Azadirachta indica	Ag NPs, 19.27-22.15 nm with spherical shape	Antiinflammatory, antidiabetic activity	Chi et al. 2022
Azadirachta indica	Ag NPs, 41-60 nm with spherical shape	Biolarvicidal activity	Poopathi et al. 2015
Boerhavia diffusa	Cu NPs, 2-10 nm with irregular spherical shape	Antibacterial activity	Vaghela et al. 2018
Catharanthus roseus	Pd NPs, 38 nm with spherical shape	Catalytic activity in dye degradation	Kalaiselvi et al. 2015
Citrus medica	Cu NPs, 10-60 nm	Antimicrobial activity	Shende et al. 2015
Curcumin	Au NPs, 45.10 nm with spherical shape	Cancer chemoperathy	Amini et al. 2023
Datura stramonium	Au NPs, 75.10-156.50 nm with spherical shape	Antifungal, antioxidant, anticoagulant, thrombolytic activity	Oladipo et al. 2020
Datura stramonium	MgO NPs, 0.17 nm with rod-like shape	Antibacterial activity	Saka et al. 2022
Echinochloa stagnina	Ag NPs, 30 nm with spherical shape	Antibacterial, cytotoxic, larvacidal activity	Shehabeldine et al. 2021
Garcinia xanthochymus	ZnO NPs, 20-30 nm with spherical shape	Antioxidant and photocatalytic activity	Nethravathi et al. 2015
Ocimum sanctum	TiO_2 NPs 75-123 nm with spherical and polygonal	Healing efficacy in diabetic wounds	Ahmad et al. 2022
Ocimum sanctum	CeO_2 NPs, 34 nm with spherical shape	Photocatalytic and antibacterial activity	Bakkiyaraj et al. 2021
Oil palm	Ag NPs, 18-20 nm with spherical	Antimicrobial	Torres et al. 2021
Phyllanthus emblica	Ag NPs, 19.8-92.8 with spherical shape	Antibacterial and biofilm inhibition activity	Masum et al. 2019
Phyllanthus emblica	Au NPs, 5-60 nm with circular, triangular, and polygonal shape	Potential anticancer activity	Wang et al. 2021
Phyllanthus emblica	Ag NPs, 4-5 nm with spherical shape	Biogenic reducing agent, antioxidant and antibacterial activity	Suvandee et al. 2022
Piper nigrum	Ag NPs, 15-38 nm with spherical shape	Antibacterial, cytotoxicity	Kanniah et al. 2021
Sambucus ebulus	Ag NPs, 35-50 nm with spherical shape	Catalytic, antibacterial, anticancer	Hashemi et al. 2022
Sugar cane leaves	Ag NPs, 16.90 nm with cubic shape	Ammonia hydrogen peroxide detection	Srikhao et al. 2021
Tamarindus indica	Ag NPs, 20-52 nm with spherical shape	Anticancer activity	Gomathi et al. 2020
Tamarindus indica	Cu NPs, 88 nm with spherical shape	Catalytic and antibacterial activity	Ashok et al. 2020
Tamarindus indica	Carbon nanotubes, 18-65 nm with microstructure sheets with thickness	Supercapacitor	Thirumal et al. 2021
Tinospora cordifoli	Se NPs, 100-200 nm with spherical shape	Antioxidant and Antiproliferative activity	Puri & Patil 2022
Tinospora cordifoli	SnO_2 NPs, 6.90-28.40 nm with non-uniform spherical shape	Photocatalytic activity for rhodamine dye degradation	Fatimah et al. 2022

2.2. Nanoparticle synthesis by microorganisms

Microorganisms are present all over the world, such as in plants, soil, water, and air. However, certain microorganisms, which can cause infections that are harmful to humans, while some microorganisms are significant in maintaining human health and are known as beneficial microorganisms. Microorganisms such as bacteria, fungi, viruses, and microalgae have been used for the green synthesis of NPs like plant-based sources (Shah et al. 2015; Gahlawat & Choudhury 2019; Jeevanandam et al. 2022). The microbial synthesis of NPs possesses several significant advantages, and they have defined morphology, size, and chemical composition, can scale up, are adaptable to various environmental conditions, and are also easy to cultivate and handle the microbial cells (Grasso et al. 2020; Jacob et al. 2021). It has also been reported that microorganisms such as *Pseudomonas aeruginosa*, *P. deceptionensis*, *Bacillus licheniformis*, *Pyrobaculum islandicum*, *Pyrococcus furiosus*, *Bacillus* sp., *Fusarium oxysporum*, *Lactobacillus* sp., *Saccharomyces cerevisiae,* and *Shewanella oneidensis* can be used for the green synthesis of NPs such as Au, Ag, Cu, Fe, Mn, Ni, Ti, and Zn (Shamaila et al. 2016; Sanaeimehr et al. 2018). However, the properties (such as size, morphology, and characteristics) of the NPs could be changed depending on the conditions of the synthesis process (temperature, pH, incubation period, etc.) (Jeevanandam et al. 2022).

The microbial synthesis of NPs is generally performed by the internalization of metal ions, which settle on the cell surface (described as the extracellular approach) or form inside the microbial cells (described as the intracellular approach). The reduction reaction of metal ions on the cell surface or inside the microbial cells may be catalyzed by the microbial enzymes, and so metallic NPs form (Salunke et al. 2016; Barabadi et al. 2017; Grasso et al. 2020). These synthesis methods of NPs described as the extracellular and intracellular approaches mainly include oxidoreductase enzymes like NADPH-dependent sulfite reductase and NADH-dependent nitrate reductase and factor cellular transporters. The extracellular approach ensues exterior to the microbial cell after utilizing various systems and these are (1) the microbial biomass, (2) the supernatant of microbial cultures, and (3) cell-free extracts (Al-Khattaf 2021) (Figure 2). The intracellular process to synthesize NPs involves supplementary phases to isolate the synthesized and gathered NPs from bacterial cells or cell biomass and, so is less favored (Annamalai et al. 2021). For example, in the first approach, Ag NPs fabricated from microbial supernatant can surround constituents of the media, in this way their colloidal dispersion, characterization, and retrieval could be impeded. Despite that, this method verifies the complete elimination of bacterial biomass and the organic media constituents, however, the incubation of bacteria in distilled water for a long period may be inactivated biomolecules, especially enzymes (Annamalai et al. 2021). For NP synthesis, it is suggested as an efficient method that well-grown microorganisms are washed, centrifuged, and then sonicated in ice. In this regard, it is stated that cell-free extract or supernatant is used as a highly concentrated and suitable source for organic molecules and biomolecules such as reductive enzymes (Gholami-Shabani et al. 2015; Wadhwani et al. 2018). With this technique, the eradication of organic media constituents and bacterial biomass by frequent washing is provided to protect biomolecules against proteolytic degradation and denaturation. In this way, it can be stated that the usage of organic biomolecules is more suitable to synthesize the NPs (Badoei-dalfard et al. 2019). In this scope, many enzymes such as alpha-amylase, keratinase, laccase, nitrate reductase, protease, and xylanase, have been used to synthesize Ag NPs and these NPs demonstrated important properties, such as antiradical, anticoagulant, larvicidal, and thrombolytic activities (Lateef et al. 2015a, 2105b; Adelere & Lateef 2016; Elegbede et al. 2018a, 2018b).

Bacteria are one of the prokaryotes, and they are unicellular organisms with a simple structure. Green synthesis of several metallic ions to produce different shapes and sizes of NPs has been attained via different kinds of bacteria strains such as *Lactobacillus*, *Bacillus*, *Pseudomonas*, *Streptomyces*, *Klebsiella*, *Enterobacter*, *Escherichia*, *Aeromonas*, *Brevibacterium*, *Corynebacterium*, *Desulfovibrio*, *Plectonemaboryanum*, *Rhodobacter*, *Rhodococcus*, *Rhodopseudomonas*, *Shewanella*, and *Weissella* (Singh et al. 2016b). Generally, pH, salt concentration, and temperature can largely affect the characteristics of bacterial NP synthesis. For example, Saifuddin et al. (2009) demonstrate the synthesis of relatively smaller Ag NPs (5-50 nm) by *B. subtilis* at 37 °C and pH 7.5, which suggests that a higher incubation temperature might facilitate a more rapid reduction of Ag ions, leading to smaller NP formation. On the other hand, Das et al. (2014) observed larger size range (42-94 nm) at lower temperature of 25 °C, indicating that the rate of NP synthesis and the resulting NP characteristics can be strongly influenced by lower temperatures. These findings emphasize the need for a careful optimization of synthesis parameters to achieve desired NP sizes and properties for specific applications. Additionally, Sinha & Khare (2011) reported that mercury (Hg) NPs intracellularly synthesized by *Enterobacter* sp. were uniform in size (2-5 nm), spherical, and monodispersed in low amounts at pH 8. Also, it has been demonstrated that *Enterobacter* sp. can be used in the decontamination of the environment polluted with toxic Hg (Sinha & Khare 2011). When compared to other microbial systems, such as those used for the synthesis of Ag or Au NPs, the unique ability of *Enterobacter* sp. to synthesize Hg NPs in a controlled manner demonstrates the organism's specialized adaptation for dealing with heavy metal contamination. While other bacteria, like *Bacillus* sp., have been shown to synthesize larger and more variable NPs (e.g., Ag NPs with sizes ranging from 5-50 nm, with some studies reporting sizes up to 94 nm) (Saifuddin et al. 2009; Das et al. 2014), *Enterobacter* sp. excels in producing smaller, uniform particles (Sinha & Khare 2011). This variability in NP size may influence their applicability in specific fields, as uniform and smaller NPs are often preferred for certain biomedical and environmental applications. These findings underscore that different bacterial species possess distinct NP synthesis capabilities, influenced by their unique metabolic pathways and environmental adaptations. Such diversity suggests that tailoring the choice of microbial species for NP synthesis can optimize the properties of the NPs for specific applications, enhancing their efficacy and functionality. Different NPs synthesized from a wide range of bacteria, and their characteristics (size, morphology, and/or characteristic properties) were summarized in Table 2.

Table 2- Nanoparticle synthesis by bacteria and their characteristics

Microorganisms	*Sources*	*Method*	*NP/Size/Shape*	*Characteristics*	*References*
Halomonas elongate IBRC-M 10214	-*	Extracellular	CuO NPs, 57-79 nm with rectangular shape	Antimicrobial activity against *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 43300	Rad et al. 2018
Bacillus cereus HMH1	Soil	Extracellular	FeO NPs, 18.8–28.3 nm with spherical shape	Anticancer effects against the MCF-7 (breast cancer) and 3T3 (mouse fibroblast) cell lines	Fatemi et al. 2018
Bacillus subtilis ZBP4	Soil	Extracellular	ZnO NPs, 22-59 nm with spherical or pseudo-spherical shape	Antimicrobial activity against *Bacillus cereus*, *Escherichia coli* O157:H7, *Escherichia coli* Type 1, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, and *Staphylococcus aureus*	Hamk et al. 2023
Escherichia coli E-30, *Klebsiella pneumoniae* K-6	Stool	Extracellular	CdS NPs, from *E. coli*, 3.2-44.9 nm with spherical shape *K. pnemoniae*, 8.5-44.9 nm with spherical shape	Antimicrobial activity against *Aspergillus fumigatus*, *Geotricum candidum*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*	Abd Elsalam et al. 2018
Idiomarina sp. PR58-8	Soil	Intracellular	Lead (IV) Sulfide (PbS_2), with a tetragonal crystal lattice, a crystallite domain size of 2.38 nm, spherical shape, with an average size 6 nm	Noncytotoxic, potential for *in situ* bioimaging applications	Srivastava & Kowshik 2017
Escherichia coli sa2, *Exiguobacterium aurantiacumm* sa3, and *Brevundimonas diminuta* sa4	Soil	Extracellular	Ag NPs, 5-50 nm with spherical shape	Antimicrobial activity against *Bacillus subtilis*, *Bacillus cereus*, methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus*, *Salmonella typhi*, and *Enterobacter vermicularis*	Saeed et al. 2020
Alcaligenes faecalis GH3	Poultry soil	Intracellular	Ag NPs, 32-49 nm, spherical shape	Antibacterial activity against *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus,* antioxidant activity	Badoei-dalfard et al. 2019
Bacillus endophyticus SCU-L	Saline soil	Extracellular	Ag NPs, 5-35 nm with spherical shape	Antibacterial activity against *Candida albicans*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*	Gan et al. 2018
Bacillus cereus SZT1	Soil	Extracellular	Ag NPs, 18-39 nm with spherical shape	Antibacterial activity *in vitro* and *in vivo* against rice bacterial pathogen *Xanthomonas oryzae* pv. *oryzae*, no toxicity to healthy rice plants	Ahmed et al. 2020
Streptococcus griseoplanus SAI-25	Rhizospheric soil of rice	Extracellular	Ag NPs, 19.5-20.9 nm with spherical shape	Antifungal activity against charcoal rot pathogen *Macrophomina phaseolina*	Vijayabharathi et al. 2018
Shewanella loihica PV-4	-	Intracellular/ Extracellular	Au, Pd, and Pt NPs, 2-7 nm, spherical	Catalytic activity on the degradation of methyl orange dye	Ahmed et al. 2018
Pseudomonas poae CO	Garlic plants	Extracellular	Ag NPs, 19.8-44.9 nm with spherical shape	Antifungal activity against *Fusarium graminearum*, inhibitive effect on spore germination, germ tube growth, mycotoxin production, and the damage of cell membrane of *Fusarium graminearum*	Ibrahim et al. 2020
Micrococcus yunanensis J2	Sarcheshme mine soil	Extracellular	Au NPs, 15-55 nm, spherical shape	Antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Klebsilella pneumoniae*, and cytotoxicity effect on six cancer cell lines of U87, HT1080, PC12, Caco2, MCF7, and A549	Jafari et al. 2018
Listeria monocytogenes J0161, *Bacillus subtilius* ATCC 11774 and *Streptomyces anulatus* MTCC 2528	-	Extracellular	Ag NPs, *L. monocytogenes*, 62.07 nm with rod and 29.64 nm with spherical shape, *B. subtilis*, 143.60 nm with hexagonal, 76.38 nm with spherical shape, S. anulatus, 42.44 nm with rod, 37.96 nm with hexagonal, 28.99 nm with triangular and 11.53 nm with spherical shape	Antifungal activity against the entomopathogenic fungus *Chrysosporium keratinophilum* MTCC 2828, the larvae, pupae and adults of *Anopheles stephensi* and *Culex quinquefasciatus* were susceptible to Ag NPs	Soni & Prakash 2015
Weissella oryzae DC6	Mountain ginseng	Intracellular	Ag NPs, 10-30 nm with spherical shape	Antimicrobial activity against clinical pathogens including *Vibrio parahaemolyticus*, *Bacillus cereus*, *Bacillus anthracis*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*	Singh et al. 2016a

*-: not described.

In NP synthesis with the intracellular approach by mold and yeasts, metal ions internalized within the fungal mycelia are reduced to less toxic forms, whereas the extracellular approach involves bioactive metabolites and enzymatic secretions facilitating NP formation (Molnár et al. 2018; Zhao et al. 2018; Rajeshkumar & Sivapriya 2020). Compared to bacteria, molds and yeasts exhibit distinct advantages in NP biosynthesis, including the production of diverse bioactive metabolites, improved production, and higher aggregation efficiency (Castro-Longoria et al. 2011; Alghuthaymi et al. 2015). However, bacterial systems offer superior genetic manipulability, faster growth rates, and more precise control over NP morphology, which are critical factors in biomedical applications (Jeevanandam et al. 2016; Shamaila et al. 2016). Furthermore, different yeast strains vary in their NP biosynthesis efficiency. For instance, yeast strains such as *Candida glabrata* and *Schizosaccharomyces pombe* have been reported the intracellular synthesis of cadmium sulfide (CdS), Ag, Se, Ti, and Au NPs, with enhanced biocompatibility and stability (Moghaddam et al. 2015; Feng et al. 2017). Conversely, certain yeast species predominantly support extracellular NP formation, which facilitates large-scale synthesis and industrial applicability. Also, NPs had various morphology (at 2-16 nm sizes with rounded, spherical, or oval shapes) were obtained by using different molds and yeasts such as *Alternata alternate*, *Aspergillus oryzae*, *Colletotrichum* sp., *Fusarium oxysporum*, *Verticillium luteoalbum*, *Trichothecium* sp., *Trichoderma viride*, *Candida glabrata*, *C. albicans*, *Rhodotorula glutinis*, and *R. mucilaginosa* (Jeevanandam et al. 2016; Ananthi et al. 2018; Cunha et al. 2018; Jalal et al. 2018; Dhabalia et al. 2020). NPs synthesized by different kinds of fungi and their various characteristics such as size, morphology, and/or characteristic properties were summarized in Table 3.

Table 3- Nanoparticle synthesis by fungi and their characteristics

	Sources	***Method***	***NP/Size/Shape***	***Characteristics***	***References***
Mold					
Trichoderma longibrachiatum	Cucumber	Extracellular	Ag NPs, 5-25 nm with spherical shape	Antifungal against phyto-pathogenic fungi: *Alternaria alternate*, *Fusarium oxysporum*, *Fusarium verticillioides*, *Fusarium moniliforme*, *Aspergillus flavus*, *Aspergillus heteromorphus*, *Penicillium glabrum*, *Penicillium brevicompactum*, *Pyricularia grisea*, and *Helminthosporium oryzae*	Elamawi et al. 2018
Penicillium oxalicum	-*	Extracellular	Ag NPs, 60-80 nm with spherical shape	Antibacterial activity against against *Staphylococcus aureus*, *Shigella dysenteriae*, and *Salmonella typhi*	Feroze et al. 2020
Aspergillus niger	-	Extracellular	Ag NPs, 25.5-543.3 nm with spherical shape	Antifungal effect on *Aspergillus niger*, and *Aspergillus flavus*	Gursoy 2020
Cladosporium perangustum DflLAKPAG05	Parasitic plant *Dendrophthoe falcata* grown on mango plant	Extracellular	Ag NPs, 30-40 nm with spherical shape	Antioxidant activity, anticancer activity on MCF-7 (human breast adenocarcinoma cells) cancer cell line and nano-toxicology activity on human blood RBCs and green gram seedling growth of *Vigna radiata*	Govindappa et al. 2020
Cladosporium sp. MK138585	*Commiphora wightii*	Extracellular	Au NPs, 5-10 nm with spherical shape	Photocatalytic activity for the degradation of Rhodamine-B (Rh-B) and Methylene Blue (MB) under sunlight irradiation, non-toxic on normal cell lines (3T3-L1), *in vitro* cytotoxicity against MCF-7 cancer cell line and *in vivo* inhibitive effect on tumor growth in ascitic tumor model	Munawer et al. 2020
Fusarium oxysporum	-	Extracellular	Pt NPs, 25 nm with face-centered cubic shape	Antioxidant and antibacterial activity against *Escherichia coli*	Gupta & Chundawat 2019
Fusarium oxysporum UTMC-5026	-	Extracellular	Au NPs, 22-30 nm with spherical and hexagonal shapes	Antibacterial activity against *Bacillus cereus* PTCC-1247, *Escherichia coli* PTCC-1270, *Pseudomonas aeruginosa* PTCC-1310, *Staphyloccoccus aureus* PTCC-1179	Naimi-Shamel et al. 2019
Penicillium chrysogenum	-	Extracellular	Pt NPs, 5-40 nm with spherical shape	Cytotoxicity activity toward myoblast C2C12 carcinoma cells via reactive oxygen species (ROS)-mediated nucleus NF-κB and caspases activation	Subramaniyan et al. 2018
Yeasts					
Yarrowia lipolytica DSM 3286	-	Intracellular	Ag NPs, 12.4-24 nm with spherical shape	Antibacterial activity against *Staphyloccocus aureus*, *Eshcerichia coli, Proteus vulgaris*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*	Bolbanabad et al. 2020
Candida albicans C86		Extracellular	Ag NPs, 30-70 nm with spherical shape	Antifungal activity against *Candida* strain C403	Dhabalia et al. 2020
C. albicans	Patients at Al-Elweya children's	Extracellular	Ag NPs, 40.19 nm with spherical shape	Antioxidant activity, cytotoxic action against HT-29 cancer cells, non-toxic against normal cell line	Hikmet & Hussein 2021
Candida glabrata	Oropharyngeal mucosa of patients	Extracellular	Ag NPs, 2-15 nm with spherical and oval shapes	Antimicrobial activity against *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneomonaie*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Candida dubliniensis*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, and *Candida glabrata*	Jalal et al. 2018
Rhodotorula glutinis and *Rhodotorula mucilaginosa*	Soil	Intracellular	Ag NPs, *R. glutinis*, 15.45 nm, *R. mucilaginosa*, 13.70 nm with rounded shapes	Antifungal activity against *Candida parapsilosis*, catalytic capacity in the degradation of 4-nitrophenol and methylene blue and cytotoxicity activity on HK-2 (human kidney cells)	Cunha et al. 2018
Filobasidium stepposum	Soil	Intracellular	Cu-In-S (in the presence of copper, indium, and sülfür) NPs, 3-5 nm with triangular shape	Photostability, showing potential as photosensitizers in solar cells	Arriaza-Echanes et al. 2024

*-: not described

Algae are autotrophic organisms, which can easily develop with minimal environmental conditions. They act as a “nano factory” for NP synthesis because of their secondary metabolites and many physiologically active substances that serve as capping agents during the synthesis (Fawcett et al. 2017). Previously, da Silva Ferreira et al. (2017) used dry biomass of *Chlorella* *vulgaris* for NP synthesis, AgCl-NPs (ranging from 10 to 80 nm and spherical shape) from *C. vulgaris* had antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumonia*. In the other studies, Bhuyar et al. (2020) synthesized Ag NPs ranging between 25-60 nm sizes using the marine microalga *Padina* sp., and Ag NPs showed antimicrobial activity against *P. aeruginosa* and *S. aureus*, while Fatima et al. (2020) synthesized Ag NPs with a size range of 60-70 nm and spherical shape using the red algae *Portieria hornemannii* and these NPs showed antimicrobial activity against the fish pathogens *Vibrio harveyii*, *V. parahaemolyticus*, *V. alginolyticus*, and *V. anguillarum*. Besides, various microalgae strains such as *Cystoseira trinodis*, *Sargassum tenerrimum*, *Sargassum ilicifolium* and *Turbinaria conoides* have been reported to synthesize Au, CuO, and AlO NPs (Ramakrishna et al. 2016; Gu et al. 2018; Koopi & Buazar 2018). However, microalgae-based NP synthesis often faces challenges related to extended synthesis durations and less precise control over particle size distribution when compared to bacterial systems. This is primarily due to the complex composition of microalgae biomolecules, which can lead to variability in NP characteristics. In contrast, bacterial-based synthesis can offer more controlled environments, resulting in more uniform NP sizes (Chugh et al. 2021; Kulkarni et al. 2023). NPs synthesized by microalgae and their characteristic properties were given in Table 4.

Table 4- Nanoparticle synthesis by microalgae and their characteristics

Microorganisms	***Sources***	***Method***	***NP/Size/Shape***	***Characteristics***	***References***
Amphiroa rigida	Coastal area of Kanyakumari District	Extracellular	Ag NPs, 20-30 nm with spherical shape	Antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, cytotoxicity on breast cancer cells (MCF-7) and larvicidal efficiency against the 3rd and 4th instar larvae of *Aedes aegypti*	Gopu et al. 2021
Chlorella ellipsoidea	Domestic sewage water of Silchar town (Assam, India)	Intracellular	Ag NPs, 220.8 nm with mostly spherical shape	Photocatalysts for degradation of water-soluble pollutants, methylene blue and methyl orange dye and antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*	Borah et al. 2020
Cystoseira baccata	lower intertidal rocky shore in the NW coast of Spain	Extracellular	Au NPs, 8.40 nm with spherical shape	The cytotoxic activity against two of the human colon cancer cell lines, Caco-2 and HT-29, and on the healthy cell line PCS-201-010 CRC	González-Ballesteros et al. 2017
Spirulina platensis	Freshwater canals near Shebin El-Kom, Menoufia Government, Egypt	Intracellular	Au NPs, 15.60-77.13 nm with octahedral, pentagonal and triangular shape	Antiviral activity against HSV-1 on Vero cells	El-Sheekh et al. 2022
Oscillatoria sp.	Freshwater canals near Shebin El-Kom, Menoufia Government, Egypt	Intracellular	AgO NPs, 14.42 to 48.97 nm with spherical shape	Antiviral activity against HSV-1 on Vero cells	El-Sheekh et al. 2022
Spirulina platensis	-	Intracellular	Pd NPs, 10-20 nm with spherical shape	Adsorbent for lead removal from aqueous solution	Sayadi et al. 2018
Synechococcus moorigangae InaCC M208	Jakarta Bay, Indonesia	Intracellular	Au NPs, 2.5 to 19.9 nm with spherical shape	Antimicrobial activity against *Staphylococcus aureus* B4, *Escherichia coli* B5, *Klebsiella pneumoniae* 1617, *Pseudomonas aeruginosa* N90PS, and *Escherichia coli* 8654, antioxidant activity	Purbani et al. 2024
Phaeodactylum tricornutum	-	Extracellular	Ti NPs NPs 50-200 nm, Spherical shape	Antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, *in vitro* cytotoxicity against human alveolar adenocarcinoma (A549), human prostate adenocarcinoma (PC-3), human mammary gland adenocarcinoma (MDA-MB) cancer cell lines and human neonatal fibroblasts (CCD34LU) non-cancerous cell lines	Caliskan et al. 2022

*-: not described.

Viruses are also non-cellular parasites (nanosized) that can infect host cells (including animals, bacteria, fungi, humans, and plants) by delivering their genetic materials. The general sizes of most viral capsids are between 20 and 500 nm in diameter and viruses are considered natural NPs because of their sizes (Soto & Ratna 2010; Jeevanandam et al. 2019). Viruses, on the other hand, serve as naturally occurring nanoscale structures with well-defined morphological and structural properties, making them promising candidates for nanotechnology applications (Jeevanandam et al. 2019). Their inherent self-assembling capabilities and biocompatibility have facilitated their utilization in biomedical applications such as gene and drug delivery, vaccines/immunotherapeutics, and theranostics and imaging (Ghosh et al. 2021). Plant viruses exist morphologically in various forms, generally in the form of rigid rods (helical) and roughly spherical (icosahedral). Frequently used icosahedral viruses in nanotechnology are the Brome mosaic virus, cowpea chlorotic mottle virus, and cowpea mosaic virus. In contrast, the most frequently utilized rod-shaped virus is the tobacco mosaic virus (Wen & Steinmetz 2016; Zhang et al. 2018). However, the major limitation of virus-based NP synthesis is their potential immunogenicity, which may pose biosafety concerns for clinical applications (Nooraei et al. 2021).

On the other hand, all these microbial-based NPs with antimicrobial, anticancer, antioxidant, and catalytic properties have been used in various fields. Notably, Au NPs synthesized using *Penicillium aculeatum* have demonstrated promising scolicidal agent against *Echinococcus granulosus*, indicating potential applications in the treatment of cystic hydatid disease (Barabadi et al. 2017). Similarly, Ag NPs obtained from *Cryptococcus laurentii* had anticancer activity against breast cancer cell lines, and the reason that the stimulation of apoptosis, sustainability, and endocytic action of tumor cell lines was influenced via synthesized Ag NPs (Ortega et al. 2015). Another study conducted by Pugazhendhi et al. (2018) also stated the potential of microbial-based NPs, where they synthesized Ag NPs by marine red algea, *Gelidium amansii* and determined that these NPs can show antifouling characteristics at an important level due to their bactericidal activities against biofilm-forming pathogens. Additionally, hybrid approaches involving different bacterial consortia have shown enhanced NP synthesis efficiency and bioactivity. In this regard, Ag NPs synthesized using a mixture of *Lactobacillus* sp. isolated from raw milk and *Bacillus* sp. isolated from soil exhibited superior antimicrobial efficacy against *S. aureus* and *P. aeruginosa* compared to NPs synthesized by either bacterium alone, highlighting the potential advantages of synergistic microbial systems (Al-Asbahi et al. 2024).

Generally, different types of microorganism species such as *Bacillus*, *Halomonas*, *Escherichia*, *Klebsiella*, *Alcaligenes*, *Pseudomonas*, *Weissella*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Candida*, *Rhodotorula*, *Amphiroa*, and *Spirulina* have been successfully used to NP synthesis such as Ag, Au, Pd, CdS, ZnO, FeO NPs with antimicrobial, anticancer, antioxidant, and catalytic activity. Although these studies were performed, more research about using microbial synthesis of NPs in various diagnostics would provide a more feasible perspective in the future, especially *in vivo* studies.

2.3. Nanoparticle synthesis by beneficial microorganisms

Beneficial microorganisms, which positively affect human health, include various species such as lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeasts that play a role in the production of fermented foods, especially probiotics (Hill et al. 2014; De Bellis & Rizzello 2024). The investigation of the beneficial microorganisms as green synthesis is important for nanobiotechnological promises due to their nontoxic and/or environmentally friendly properties. For this scope, NPs synthesized by beneficial microorganisms can exhibit diverse therapeutic applications, such as antimicrobial, antioxidant, catalytic, anticancer, and cytoprotective activities (Ghosh et al. 2022).

LABs are Gram-positive and generally accepted as probiotics, they have thick cell walls consisting of glycosides, peptides, polysaccharides, and proteins. These structures are known as bioreduction sites and attract metal ions because of their negative electric potential, this property facilitates NP synthesis and serves as a protective mechanism against metal toxicity (Król et al. 2018; Mohd Yusof et al. 2019). Various NPs, including Ag, Se, Au, and ZnO, have been synthesized using LAB strains (Nair & Pradeep 2002; Bandeira et al. 2020; Spyridopoulou et al. 2021). For example, Ag NPs synthesized by *Lactiplantibacillus plantarum* HDL-03 had crystalline and metallic properties, with the particle sizes of 36.63 nm, and antimicrobial activity against *B. subtilis*, *M. luteus*, *E. coli*, *S. aureus*, *S. enterica*, and *S. paratyphi*. The results showed that Ag NPs synthesized by LAB can be used as antimicrobial agents at safe biological concentrations (Zang et al. 2024). Similarly, *Lactobacillus acidophilus* HN23 was used to synthesize Se NPs, yielding particles ranging from 60 to 300 nm. Compared to Se, these Se NPs exhibited significantly lower toxicity and demonstrated therapeutic potential in mitigating lipid accumulation and enhancing mitochondrial membrane potential in WRL68 human hepatic cells, indicating their potential application in treating nonalcoholic fatty liver disease (Lei et al. 2024). Additionally, *Lacticaseibacillus casei* ATCC 393 assisted synthesis of Se NPs exhibited *in vitro* antiproliferative activity, induced apoptosis, and increased ROS levels in BALB/c mice's CT26 syngeneic colorectal cancer cell models (Spyridopoulou et al. 2021). On the other hand, ZnO NPs synthesized using probiotic bacteria (*Limosilactobacillus fermentum*) exhibited an average size between 100 and 120 nm, with pronounced antimicrobial activity, especially against *Vibrio harveyi* (Shanmugam et al. 2023). Different NPs synthesized by LAB, and their characteristic properties such as size, morphology, and/or various activity were summarized in Table 5.

AAB are Gram-negative α-Proteobacteria, which are obligate aerobes. They are found commonly in environments such as fruits, flowers, rotten fruits or flowers, and so on they are well known for their ability to oxidize sugars and alcohols into organic acids, which has been utilized in the production of fermented foods such as vinegar, Kombucha, lambic beer, and water kefir, all of which possess various health benefits (Buddhika et al. 2021; Hata et al. 2023). Additionally, AAB can produce exopolysaccharides that received significant attention in designing hydrogel materials with applications in tissue engineering for their viscoelasticity, swelling, morphology, and thermal stability, these exopolysaccharides commonly referred to as bacterial cellulose, have also been investigated in NP synthesis (Qi et al. 2020; Gupte et al. 2021). A notable example includes a study utilizing *Glucanoacetobacter kombuchae* to synthesize Ag NPs. Results showed that NPs were 20 nm with spherical shapes and demonstrated potent antioxidant and antimicrobial activity against *B. cereus*, *E. coli*, *S. aureus*, *S. sonnei*, *S. typhi*, and *V.*

cholerae. Besides, the anticancer effect of NPs detected on HEPG2 hepatoblastoma, MCF-7 breast, and MDA-MB 468 triple-negative breast cancer cell lines (Majumder et al. 2022). Similarly, *Acetobacter xylinum* NCIM 2526 produced Au NPs with excellent catalytic activity in the sodium borohydride-mediated reduction of 4-nitro phenol and methylene blue, demonstrating the potential of AAB-derived NPs in catalytic applications (Ahmed et al. 2014).

Table 5- Nanoparticle synthesis by lactic acid bacteria and their characteristics

Microorganisms	*Sources*	*Method*	*NP/Size/Shape*	*Characteristics*	*References*
Bifidobacterium bifidum	Patients with diarrhea who had known clinical symptoms	Extracellular	TiO_2 NPs, 81 nm with spherical or oval	Antimicrobial activity against *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Klebsiella pneumoni*, *Escherichia coli* and *Salmonella typhi*	Ibrahem et al. 2020
Lactobacillus kimchicus (*Secundilactobacillus kimchicus*) DCY51	Korean kimchi	Intracellular	Au NPs, 5-30 nm with spherical shape	Antioxidant activity, *in vitro* low toxicity in murine macrophage (RAW264.7) and human colon cancer cell lines (HT29)	Markus et al. 2016
Lactobacillus sp.	Raw milk	Intracellular	Ag NPs, 11-22.8 nm, spherical shape	Antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and non-hemolysis effect	Al-Asbahi et al. 2024
Lactobacillus sporogenes	-	Intracellular	ZnO NPs, 145.70 nm with hexagonal shape	Antimicrobial activity against *Staphylococcus aureus*	Mishra et al. 2013
Lactiplantibacillus plantarum VITES07	Curd sample (fermented milk product)	Intracellular	ZnO NPs, 7-19 nm with roughly spherical shape	Potential non-toxic	Selvarajan & Mohanasrinivasan 2013
Lactobacillus sp.	-	Extracellular	TiO_2 NPs, 50-100 nm, spherical shape	Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, less haemolytic activity compared to the antibiotic ampicillin	Ahmad et al. 2014
Lactobacillus acidophilus	Fermented milk products	Intracellular	Ag NPs, 19-25 nm with spherical shape	Antimicrobial activity against *Escherichia coli* ATCC25,922, *Pseudomonasaeruginosa* AT CCC 27,853, and *Salmonella enterica* ATTC 14,028, Gram-positive: *Staphylococcus aureus* ATCC 29,213 and *Bacillus subtilis* ATCC 6051, and fungus *Candida albicans* ATCC 10,231 and cytotoxic effect on A549 (Lung), Caco (Colon), and HepG2 (Liver) cancer cell lines	Mohammed et al. 2023
Lactobacillus sp.	Fermented food product	Extracellular	Fe_2O_3 NPs, 31.34 nm with spherical shape	Antibacterial activity on *Pseudomonas aeruginosa*	Abd Qasim & Yaaqoob 2023
Lactobacillus sp. strain LCM5	Brined cucumbers	Extracellular	Ag NPs, 3-35 nm with spherical shape	Antimicrobial activity against *Aspergillus ochraceus*, *Aspergillus flavus*, *Penicillium expansum*, and *Chromobacterium violaceum*	Matei et al. 2020
Lactobacillus acidophilus	Mother dairy's curd	Extracellular	Se NPs, 2-15 nm with spherical shape	Antibacterial activity on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, cytotoxicity activity against HEK-293 (Human Embryonic Kidney) cell lines	Alam et al. 2020
Lactobacillus sp.	Cow milk	Intracellular	ZnO NPs, 32 nm with spherical shape	Antimicrobial activity against *Clostridium difficile*, *Clostridium perfringens*, *Esherichia coli*, *Salmonella typhi*, *Candida albicans*, *Aspergillus flavus*, and biocompatibility activity against human colon cancer (HT-29) cell lines	Suba et al. 2021
Lactiplantibacillus plantarum	-	Extracellular	Ag NPs, 40-50 nm with spherical shape	Antioxidant activity, antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, and Enterococcus faecalis*	Prema et al. 2022
Lactiplantibacillus plantarum BSe	Citrus	Intracellular	Se NPs,100 nm with round shape	Antimicrobial activity against *Escherichia coli* GDMCC 1.118 and *Staphylococcus aureus* GDMCC 1.2442 and cytotoxicity on 293T cell line of human renal epithelial cells	Zhong et al. 2024

*-: not described.

Yeasts are widely used as starter culture in various food industries, such as winemaking, bakery, brewing, and dairy production, because of their desirable technological and sensory properties. The most frequently utilized yeasts in food products and supplements include *Saccharomyces cerevisiae* and *S. boulardii*, the latter being recognized as a probiotic yeast) (Chan & Liu 2022). Beyond their traditional applications, yeasts have recently been investigated as potential alternative protein sources due to their optimal amino acid composition, and their ability to be cultivated using industrial waste products, making them an eco-friendly protein source (Jach et al. 2022). On the other hand, yeasts also provide several advantages in NP synthesis, including ease of cultivation under laboratory conditions, rapid growth rates, and the ability to utilize simple nutrients, making them highly suitable for scalable and environmentally sustainable NP production (Skalickova et al. 2017). For this purpose, in a study reported by Abdulradha & Alhadrawi (2023), Zn NPs were synthesized using the probiotic *S. boulardii*, yielding spherical NPs with an average size of 24.61 nm. These Zn NPs showed antimicrobial activity against *Burkholderia cepacia*, a pathogen associated with diabetic foot ulcers. Furthermore, Pt NPs synthesized by *S. boulardii* had important anticancer activity against A-431 epidermoid squamous carcinoma and MCF-7 breast cancer cell lines (Borse et al. 2015). NPs synthesized by beneficial yeast, which are commonly used as starter cultures in the food industry, and their characteristic properties are compiled in Table 6.

Table 6- Nanoparticle synthesis by beneficial yeasts and their characteristics

Microorganisms	***Sources***	***Method***	***NP/Size/Shape***	***Characteristics***	***References***
Saccharomyces cerevisiae	Baker's yeast	Extracellular	Ag NPs, 10-60 nm with spherical shape	Antimicrobial effect against *Cryptococcus gastricus*, *Esherichia coli*, *Trichophyton rubrum*, *Shigella flexneri*, *Fusarium oxysporum*	Sowbarnika et al. 2018
Saccharomyces cerevisiae	Baker's yeast	Intracellular	Se NPs, 30-100 nm, spherical shape	Antioxidant activity	Faramarzi et al. 2020
Pichia kudriavzevii HA-NY2 and *Saccharomyces uvarum* HA-NY3	Egyptian sweet fruits	Extracellular	Ag NPs, *P. kudriavzevii*, 20.66 nm with cubic regular shape, *S. uvarum*,12.4 nm with round shape	Antimicrobial effect against *Bacillus subtilis* ATCC6633, *Staphylococcus aureus* ATCC29213, *Pseudomonas aeruginosa* ATCC27953, *Candida tropicalis* ATCC750, and *Fusarium oxysporium* NRC21, inhibiting of paw edema by oral administration, anticancer activity on HCT-116 (Colon cell line) and PC3 (Prostate cell line), no ulcerogenic effects	Ammar et al. 2021
Saccharomyces cerevisiae	Dry yeast	Intracellular	Pd NPs, 32 nm, hexagonal shape	Photocatalytic activity on degradation of the azo dye direct blue 71	Sriramulu & Sumathi 2018
Saccharomyces cerevisiae	Baker's yeast	Intracellular	TiO_2 NPs, 36-12.0 nm with spherical shape and rough surfaces	Antibacterial activity against *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231, Methicillin resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* and photocatalytic activity into methylene blue dye	Peiris et al. 2018
Saccharomyces cerevisiae	Baker's yeast	Intracellular	ZnO NPs, 13.0-20.0 nm with spherical shape	Antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, photocatalytic efficiency on degradation of Eriochrome Black T	El-Khawaga et al. 2023
Saccharomyces cerevisiae PTCC 5269	-*	Extracellular	Ag NPs, 29.07 nm with spherical shape	The antibacterial effect on *Listeria monocytogenes* ATCC 7644 and *Streptococcus marcescens* PTCC 1111, antioxidant activity, thrombolytic potential to lyse the blood clots, the ability of alpha-amylase inhibition	Barabadi et al. 2024
Saccharomyces cerevisiae	-	Extracellular	Ag NPs, 5-30 nm with spherical to oval shapes	Antibacterial properties against *Escherichia coli* (KCCM 11234) and *Staphylococcus aureus* (KCCM 11335) and enhancing seed germination, seedling vigor, and disease resistance for *Sorghum jowar* and *Zea mays* seeds	Kim et al. 2024

*-: not described

Considering the diverse applications and synthesis mechanisms of LAB, AAB, and yeasts, each microbial system exhibits distinct advantages in NP biosynthesis. LAB-mediated synthesis is characterized by the production of biofunctional NPs with antimicrobial and therapeutic properties, whereas NPs synthesized by AAB offer enhanced catalytic activity and potential in drug delivery systems. Yeasts, with their rapid growth and high biomass yield, provide a scalable platform for NP synthesis, particularly for biomedical applications. Despite these advantages, differences in NP morphology, particle size distribution, and synthesis efficiency must be considered when selecting a microbial system for NP production.

3. Potential Applications of Nanoparticles

Green synthesis technology is a promising interdisciplinary field that provides novel synthetic materials with broad application prospects in the fields of biology, agriculture, food medicine, and structural materials (Osman et al. 2024). There have been several researches on the utilization of microorganisms for NP synthesis, and microbial NPs are used for anticancer, antimicrobial, anti-biofouling, biosensor, catalysis, medical diagnosis, and mosquito larvicidal purposes (Salunke et al. 2016). Also, NPs are commonly employed in combination with traditional therapies or active substances to augment cellular permeability and effectiveness, while simultaneously reducing the negative effects and morbidity linked to advanced cancer treatment (Ma et al. 2020). Potential application fields of microbially synthesized NPs are presented in Figure 3.

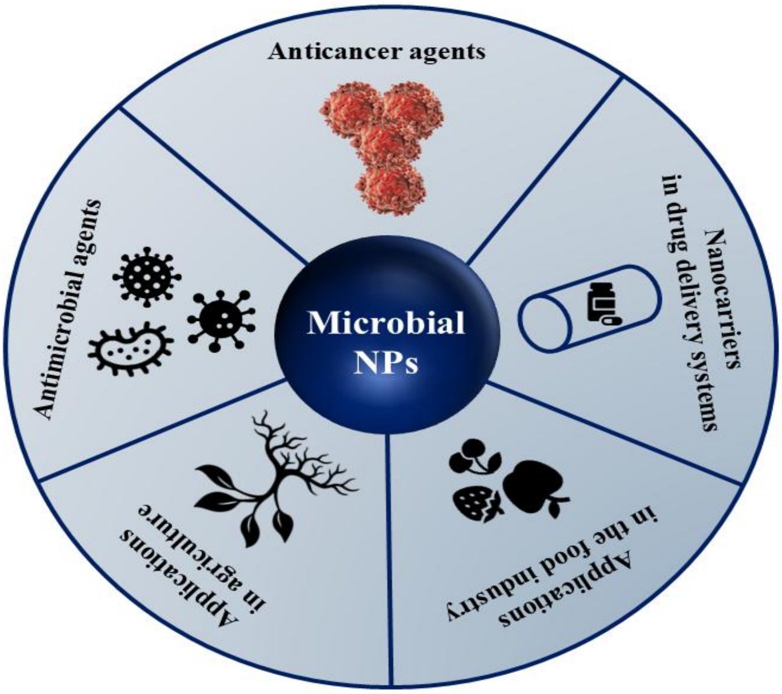

Figure 3- Schematic for the potential applications of microbial nanoparticles

3.1. NPs as antimicrobial agents

The occurrence of resistant strains because of the continuous utilization of antibiotics has dramatically increased infectious diseases. In this context, NPs have an important potential in terms of antimicrobial agents. Some physicochemical characteristics of NPs underlying their antimicrobial activities include their size, surface morphology, charge, and crystal structure (Wang et al. 2017). NPs' small size is a main advantage in achieving strong antimicrobial actions and effectively combating intracellular bacteria since it facilitates the penetration of NPs through bacterial cell walls into the bacteria (Shaikh et al. 2019). The antimicrobial effect mechanism of NPs includes the destruction of the cell wall, causing the leakage of cellular components including proteins, enzymes, DNA, and metabolites into the surrounding environment, ultimately leading to the breakdown of cellular structure (Gomaa 2017; Yuan et al. 2017; Ravichandran et al. 2018; Jalal et al. 2019). The schematic representation of the antimicrobial mechanisms of NPs is given in Figure 4. Firstly, NPs adhere to the cell surface to the plasma membrane and later on transport within the cell where they interact with DNA, so both inhibiting replication and changing the respiratory chain (Slavin et al. 2017). Several studies have investigated the antimicrobial activity of NPs synthesized by microorganisms. For example, Gan et al. (2018) reported that *B. endophyticus*, isolated from soil, extracellularly synthesized Ag NPs with spherical morphology and size range of 5-35 nm, exhibiting significant antibacterial activity with inhibition zones of 15 mm against *C. albicans*, 13 mm against *E. coli*, 11 mm against *S. typhi*, and 19 mm against *S. aureus*. Similarly, *Lactobacillus* sp. (isolated from raw milk) and *Bacillus* sp. (isolated from soil) were utilized for the biosynthesis of Ag NPs. The intracellularly synthesized Ag NPs exhibited spherical morphology with sizes ranging from 4.65 to 22.8 nm. Antibacterial activity analysis demonstrated that Ag NPs derived from *Lactobacillus* sp. exhibited inhibition zones of 12 mm against *S. aureus* and 11 mm against *P. aeruginosa*, whereas those synthesized by *Bacillus* sp. showed inhibition zones of 11 mm and 10 mm, respectively (Al-Asbahi et al. 2024). Additionally, Ibrahem et al. (2020) reported that *B. bifidum*, isolated from patients with diarrhea exhibiting known clinical symptoms, extracellularly synthesized TiO_2 NPs with spherical or oval morphology and an average size of 81 nm. These TiO_2 NPs demonstrated antimicrobial activity at concentrations of 16 mg/mL and 32 mg/mL against *E. coli* and *S. typhi*, respectively.

The other antimicrobial mechanism of NPs is the occurrence of pits leading to structural damage in the cell membrane and death (Figure 4). After NPs enter the cytoplasm of microorganisms, they induce the generation of ROS, primarily through electron transfer interactions and oxidative stress. These ROS, including superoxide anions (O_2^-), hydroxyl radicals (•OH), and hydrogen peroxide (H_2O_2), cause oxidative damage to essential biomolecules such as DNA, lipids, and proteins, ultimately leading to cellular toxicity and microbial death (Abo-zeid & Williams 2020). In this scope, for example, MgO NPs have shown a broad range of toxicity against various microorganisms such as *E. coli*, *S. aureus*, *Aspergillus niger*, and *Penicillium oxalicum* (Cai et al. 2017; Sierra-Fernandez et al. 2017). Also, NPs such as Ag, Cu, TiO_2, and ZnO are evaluated as antimicrobial agents because of the formation of ROS (e.g., hydrogen peroxide, hydroxide, or superoxide anion), which can damage cellular structure (Hemeg 2017).

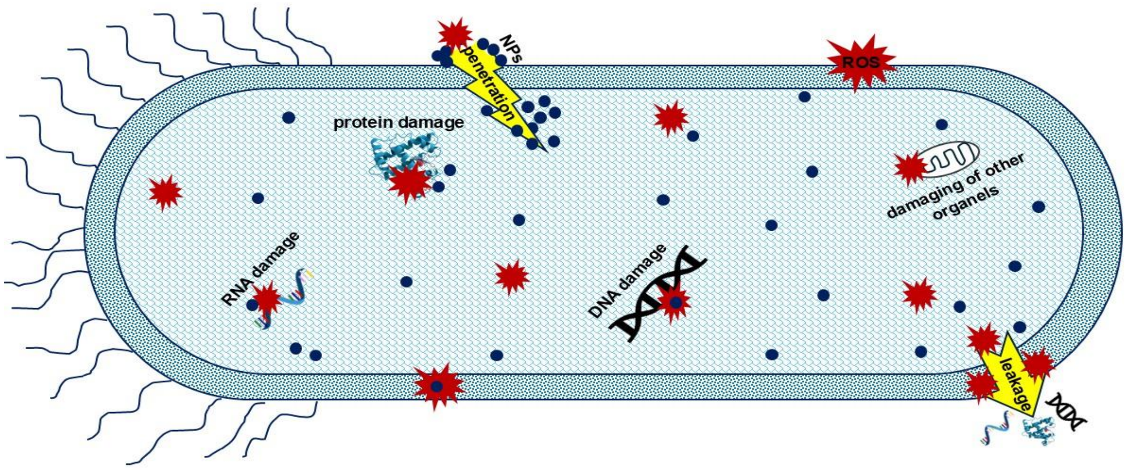

Figure 4- Schematic illustration for the inhibitory mechanisms of NPs on a microbial cell

3.2. NPs as anticancer agents

Cancer has been one of the prominent causes of mortality around the globe in recent years. It includes an assemblage of illnesses, characterized by indefinite growth and changed or no apoptosis. Generally, surgery, chemotherapy, and radiotherapy, which have undue side effects are used as methods of cancer treatment (Gegechkori et al. 2017). However, these methods are inadequate for the treatment and hence, incidences of various cancers such as blood, breast, colon, and lung are increasing each day. Hence, it is crucial to explore alternative solutions to address these challenges. According to numerous researchers, nanomedicine applications can be used in rapid tumor detection and targeted drug delivery and treatment (Bajpai et al. 2018b; Wang et al. 2020; Bloise et al. 2021; Ikram et al. 2021; Mortezaee et al. 2021; Razak et al. 2021). NPs are very comparable in size to most biological structures and compounds and thus have functional properties such as *in vivo* and *in vitro* cancer research (Oroojalian et al. 2021). Recent studies have highlighted the potential of NPs synthesized by microorganisms in cancer treatment. For example, *L. kimchicus* (*Secundilactobacillus kimchicus*) DCY51 isolated from Korean kimchi, has been utilized for the intracellular synthesis of Au NPs ranging from 5 to 30 nm in size, exhibiting spherical shape. These Au NPs have demonstrated significant antioxidant activity, as well as low toxicity in both murine macrophage (RAW264.7) and HT29 colon cancer cell lines, making them potential candidates for anticancer applications (Markus et al. 2016). Similarly, *L. acidophilus*, commonly found in fermented milk products, has been employed to synthesize Ag NPs with size range of 19 to 25 nm. The Ag NPs, also spherical in shape, displayed cytotoxic effects on several cancer cell lines, including A549 lung, Caco colon, and HepG2 liver cancer cell lines, suggesting their potential as anticancer agents (Mohammed et al. 2023). Additionally, *Lactobacillus* sp., isolated from cow milk, was used for the intracellular synthesis of ZnO NPs with spherical shape and size of approximately 32 nm. These ZnO NPs exhibited biocompatibility in HT-29 colon cancer cell lines, further supporting their role in cancer therapy (Suba et al. 2021). Moreover, yeasts such as *P. kudriavzevii* HA-NY2 and *S. uvarum* HA-NY3, isolated from Egyptian sweet fruits, have been used for the extracellular biosynthesis of Ag NPs. The Ag NPs synthesized by *P. kudriavzevii* were approximately 20.66 nm in size with cubic regular shape, whereas those synthesized by *S. uvarum* had round shape and were approximately 12.4 nm in size. These NPs exhibited potent anticancer activity against HCT-116 colon and PC3 prostate cancer cell lines (Ammar et al. 2021).

Also, it has been believed that NPs serve as tools that facilitate molecular-level interaction, and traverse biological barriers but do not affect the functioning of healthy cells. As well as their usage as a drug carrier, NPs can have an anticancer effect on their own. For instance, Au NPs promoted apoptosis in pancreatic cancer cells and reversed epithelial-mesenchymal transition, and in this way, gemcitabine-resistant cells became sensible to the drug (Qiu et al. 2018; Huai et al. 2019; Zhang et al. 2019; Tomşa et al. 2021). On the other hand, the toxicity of Au NPs can be changed depending on size itself, the larger particles with 4.8-12 nm sizes may be less toxic against healthy cells while maintaining significant toxicity against tumor cells, whereas smaller NPs with 1-2 nm sizes are toxic to both healthy and tumor cells (Liu et al. 2021; Tomşa et al. 2021). Also, Au NPs have been utilized in photothermal and photodynamic therapies for various cancer types (Liu et al. 2021). Additionally, Ag NPs can induce pro-apoptotic effects by promoting ROS production through electron transfer reactions and redox cycling processes. These mechanisms lead to oxidative stress, resulting in increased caspase-3 activity and the upregulation of the p53 protein, which collectively contribute to apoptosis (Lee & Lee 2019; Halawani et al. 2020). For example, it has been stated that the cytotoxicity of Ag NPs against MCF-7 breast cancer cells resulted from the apoptosis induction by activation of the pro-apoptotic Bax protein and the inhibition of the anti-apoptotic Bcl-2 protein, also the release of cytochrome c from mitochondria (Kulandaivelu & Gothandam 2016).

Beyond their direct cytotoxic effects, NPs can also modulate the immune response, offering additional mechanisms for cancer therapy. Microbial NPs could be loaded with immunostimulatory agents, such as cytokines, Toll-like receptor agonists, or checkpoint inhibitors, and delivered to the tumor site. These NPs can stimulate antigen-presenting cells, leading to enhanced T-cell activation and a more robust adaptive immune response against tumor cells (Chohan et al. 2023; Zeng et al. 2023; Gholami et al. 2024). In terms of cytotoxicity, Au NPs have been shown to enter human cervical cancer cells via endocytosis, where they elevate intracellular ROS, disrupt matrix metalloproteinase activity, and induce apoptosis by causing cell cycle arrest at the S-phase (Khatua et al. 2020). While these mechanisms contribute to anticancer efficacy, the potential off-target effects on healthy cells due to ROS elevation and DNA damage warrant further investigation. In addition to Au NPs, Ag NPs have been reported to induce oxidative stress and mitochondrial dysfunction, ultimately triggering apoptosis in cancer cells. Their cytotoxicity is primarily associated with the disruption of cellular membranes and increased production of ROS (Xu et al. 2023). Interestingly, the microbial NPs also demonstrated significant cytotoxicity against MCF-7 breast cancer and A549 lung carcinoma cell lines, further supporting their potential as alternative anticancer agents (Fahmy et al. 2020). However, before microbial NPs can be fully integrated into clinical practice, extensive *in vivo* studies are necessary to evaluate the long-term immunogenicity, biodistribution, and potential off-target effects (Gavas et al. 2021).

Nanoparticles synthesized from microorganisms offer a promising, environmentally friendly alternative to traditional synthetic methods. They can not only exhibit anticancer effects but also modulate immune responses, further enhancing their potential in cancer therapy.

3.3. NPs as nanocarriers in drug delivery systems

Generally, the new drug delivery systems are designed to increase therapeutic effects, but these systems should provide safe, accurate, and controlled drug delivery at the target sites. NPs are extensively used as drug delivery carriers for various therapeutic agents (nucleic acids, antibodies, chemotherapeutic drugs, etc.) (Thanki et al. 2013). Target-specific nanocarriers must traverse blood-tissue barriers to reach the target cells. These carriers enter the target cells by endocytosis and transcytosis pathways and interact with cytoplasmic constituents (Fadeel & Garcia-Bennett 2010). NP drug carriers can also pass through the blood-brain barrier and junctions of tight epithelial cells in the skin, which provides drug delivery to the desired target site. Besides, these nanocarriers can increase the biodistribution of therapeutic agents due to their high surface area-to-volume ratio and reduce toxicity at the target site (Wolfram & Ferrari 2019). For this scope, NPs like Ag, Au, Cu, Pl, TiO_2, and ZnO NPs possess improved tunable optical properties and can be used as nanocarriers. Besides, their surface can be easily functionalized to conjugate targeting agents and active biomolecules through covalent bonding, Hydrogen bonding and electrostatic interactions, and multiple drugs can be easily added to improve therapeutic efficacy (Thanki et al. 2013).

On the other hand, certain types of NPs, such as lipid-based NPs (e.g., liposomes), polymeric NPs, and micelles, increase the solubility of hydrophobic compounds, making them suitable for parenteral administration. Additionally, these NPs enhance the stability of therapeutic agents, such as oligonucleotides, peptides, and other biomolecules (Emerich & Thanos 2006). Besides, adhesion and other interfacial interactions between nanocarrier and biomembrane are necessary, especially for intracellular drug delivery (Vedernykova et al. 2018). Therefore, it is stated that drug delivery by NPs could also decrease the dosage concentration of anticancer drugs with higher specificity/efficacy and lower toxicity, like that in melanoma treatment (Cassano et al. 2021). For instance, it has been reported that ZnO NPs synthesized by *Rhodococcus pyridinivorans* loaded with anthraquinone demonstrated cytotoxicity toward colon cancer cells (HT-29 colon carcinoma) depending on the dose-dependent manner used (Kundu et al. 2014). Moreover, it was stated that NPs could show features to target tumor vessels and microenvironments and enhance the efficacy of antiangiogenic drugs (Alsaab et al. 2021). On the other hand, the study by Liu et al. (2020) demonstrated the intracellular synthesis of Au NPs by *Gluconacetobacter liquefaciens* kh-1, which served as carriers for the peptide CopA3 and ginsenoside compound K, enhancing their therapeutic efficacy. These biogenic NPs facilitated targeted drug delivery to lipopolysaccharide-activated macrophages, ensuring controlled intracellular release without significant cytotoxicity. This innovative approach showed the potential of microbial synthesized NPs as biocompatible and effective drug delivery systems in inflammation-related disorders. Additionally, the study by Pradeepa et al. (2016) introduced a novel antibiotic delivery system using Au NPs synthesized via bacterial exopolysaccharide derived from *L. plantarum*. The biogenic Au NPs were functionalized with various antibiotics, including levofloxacin, cefotaxime, ceftriaxone, and ciprofloxacin, to enhance their therapeutic efficacy against multidrug-resistant bacterial strains. Conjugation of antibiotics to Au NPs significantly improved their stability, bioavailability, and controlled release, ensuring a prolonged antibacterial effect while reducing the minimum inhibitory concentration and minimum bactericidal concentration of the drugs. Mechanistically, the Au NP-antibiotic conjugates effectively penetrated bacterial cells, overcoming resistance mechanisms such as efflux pumps and enzymatic degradation. Transmission electron microscopy analysis revealed that these NP-drug complexes disrupted bacterial membranes, leading to cytoplasmic leakage and eventual cell death. This targeted delivery system not only enhanced the antibacterial activity of conventional antibiotics but also minimized potential cytotoxic effects by reducing the required dosage. These studies not only demonstrate the therapeutic potential of NP-based drug delivery systems but also highlight the crucial role of microorganisms in the synthesis of biocompatible NPs. In both cases, *G. liquefaciens* and *L. plantarum* serve as key microbial agents, enabling the intracellular synthesis of Au NPs that have different characteristics and are functionalized for specific therapeutic applications. The use of these microorganisms ensures a sustainable, eco-friendly synthesis process and enhances the efficiency of the drug delivery system, as their metabolic pathways provide a natural method for producing NPs with tailored properties suitable for targeted drug delivery.

3.4. NPs applications in agriculture

Nanotechnology in agriculture is accepted as one of the most appropriate methods to enhance crop yield and sustain the world's continuously growing population. NPs have significant applications such as nano-fertilizers, nano-pesticides, and crop protection for the regulated dispensing of agrochemicals (de Oliveira et al. 2014; Grillo et al. 2016; Qureshi et al. 2018). The applications of NPs in agriculture as fertilizers are related to their enhanced characterization such as absorption, adhesion effects, responsiveness, and surface properties (Qureshi et al. 2018). Nanofertilizers are micro- or macro-nutrient fertilizers with a particle size of <100 nm and can be used to increase yields. They are also responsible for supplying types of nutrients (one or more) to growing plants, promoting their growth, and increasing production (Chhipa 2017). Nanofertilizers are used in two different types. Firstly, they can provide nutrients to plants to enhance their development and yield. Secondly, they are used as nutrient carriers like nanocarriers used in drug delivery systems, and they help not only in the transport and release of nutrients but also as a nutrient source (Liu & Lal 2015). However, foliar spray or irrigation can be delivered above or below ground to encourage plant growth and yield, or NPs can be added to seeds or primed (Mittal et al. 2020). The efficiency of NPs can change depending on various factors such as the plant type, as well as NP type, size, concentration, stability, chemical composition, and transformation rate after biological interaction (Prasad et al. 2017; Chen 2018).

NPs synthesized by microorganisms such as *B. subtilis*, *Paenibacillus elgii*, and *P. fluorescens* can be used as nano-biofertilizers. Since one liter of nano-biofertilizers can be applied to several hectares of crops, their requirements are so (relatively minimal in comparison with other fertilizers), making them costs-affordable. In addition to carbon-based nanomaterials, nano-clay minerals, and zeolites can also be used as fertilizers (Guo et al. 2018; Bisinoti et al. 2019). Many crops such as tomatoes, chickpeas, spinach, and maize have benefited from the use of different natural nanofertilizers such as ZnO, SiO_2, Fe slag powder, and TiO_2 NPs (Sivarethinamohan & Sujatha 2021). Additionally, the studies conducted for the potential application in food and agriculture fields of NPs are also given in Table 7.

Table 7- Potential applications in the agriculture and food fields of NPs

Applications	*NPs Type*	*Synthesis Sources*	*Properties*	*References*
Agriculture - Fertilizers	Se NPs, 50-500 nm	Extracellular synthesis by *L. acidophilus*, *L. casei*, *Bifidobacterium* sp.	Plant disease enhancer	Eszenyi et al. 2011
Agriculture-Fertilizer and Pathogen Control	Ag NPs, 12-27 nm	Extracellular synthesis by *L. casei*	Plant-growth stimulator, antimicrobial effect	Singh et al. 2015
Agriculture - Pathogen Control	Ag NPs, 20-50 nm	Extracellular synthesis by *Achromobacter* sp., *Pseudomonas* sp.	Antifungal activity against *Fusarium oxysporum* in chickpeas	Kaur et al. 2018
Agriculture - Pathogen Control	ZnO NPs, 2-83 nm	Extracellular synthesis by *Trichoderma harzianum*	Antifungal agent against soil pathogens *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium* sp.	Zaki et al. 2021
Agriculture - Pathogen Control	CuO NPs, 31-45 nm	Extracellular synthesis by *Trichoderma harzianum*	Antifungal effects against *Alternaria brassicicola* (the foliar pathogen)	Gaba et al. 2022
Agriculture - Pathogen Control	ZnO NPs, 32 nm	Extracellular synthesis by *B. subtilis*	Control growth of agricultural pathogens including *Alternaria solani, Colletotrichum capsici*, *F. oxysporum, Sclerotium rolfsii*	Nargund et al. 2022
Food Industry - Antimicrobial Packaging	Ag NPs	Cellulose and collagen	Prevent the growth of *E. coli* and *S. aureus* in sausage casings	Fedotova et al. 2010
Food Industry – Packaging	Ag NPs, 300 nm	Chitosan	Prevent the growth of *E. coli* O157: H7, *L. monocytogenes, S. aureus,* and *S. typhimurium* in food packaging	Rhim et al. 2013
Food Industry - Packaging	Ag NPs embedded in carboxymethylcellulose film	-*	Prevent the growth of *E.coli* and *E. faecalis* in food packaging	Siqueira et al. 2014
Food Industry - Food Preservation	Ag NPs, ZnO NPs, 20 nm	-	Antibacterial biodegradable films based on polylactide and polybutylene adipate terephthalate (as matrix) loaded with ZnO or Ag NPs in active packaging to preserve the fresh noodles	Yana et al. 2024

*-: not described.

3.5. NP applications in the food industry

The Food and Agriculture Organization of the United Nations highlights that over 1.3 billion metric tons of consumable food is trashed annually due to inadequate storage and transport facilities, poor post-harvest techniques, and market/consumer waste. Preventing food wastage is critical to mitigate the food crisis exacerbated by environmental challenges and growing populations (Nile et al. 2020). The primary causes of food wastage are microbial contaminations and/or spoilages, which compromise food quality and safety and increase the risk of foodborne diseases (Sperber 2009). Nanotechnology presents various solutions across the food supply chain, including production, processing, storage, and distribution. It increases food safety through nanosensors, which detect foodborne pathogens, enhance packaging with antimicrobial properties, and extend shelf life (Sekhon 2010; Nile et al. 2020). Besides, nanotechnology helps in toxin detection, color formation, and flavor production (Seklon et al. 2010). Moreover, smart nanotechnology systems provide efficient localization, sensing, and remote control of food items. Also, nanobased delivery systems can improve the nutritional value of food (Bajpai et al. 2018a). For example, carbon nanotubes serve as potent antimicrobial agents by causing cellular damage or death in *E. coli* through direct contact (Kang et al. 2007), nano-biosensors can be also used for detecting carcinogenic pathogens in food production (Nile et al. 2020). Besides, SiO_2 (E551) and TiO_2 (E171) were allowed as food additives in bulk quantities to produce healthier foods (EFSA 2009), while Ag NPs were used for the production of fortified Jambu juice to increase their content of essential vitamins and minerals (CFS 2017). Moreover, Ag NPs were utilized for sterilization, quality control, and bioavailability, supporting the immune system and defense for natural healing in supplemented functional drinks (CFS 2017). NPs are also utilized as carriers to deliver enzymes, antibrowning agents, antioxidants, flavors, etc. to develop the shelf life even if the package is opened (Cha & Chinnan 2004; Weiss et al. 2006). Fe, ZnO, C, MgO, TiO_2, and SiO_2 NPs are used as antimicrobial agents and in some conditions as food components and/or ingredients (He et al. 2019). Besides, NPs were used in various food applications, as food colorants, anticaking, hygroscopic, and drying agents, as a whitener in dairy products, and as acting antibacterial agents, absorbing and decomposing ethylene in fruit and vegetables (Mohammad et al. 2022).

On the other side, NPs are widely used in packaging systems since they show antioxidant and antimicrobial activities against the microorganisms found on the surface of food and/or food packaging materials (Wang et al. 2022). NPs (namely Ag, CuO, Cu, Fe, MgO, Pd, TiO_2, and ZnO) with antimicrobial properties that could be adhered by various interactions (such as covalent, electrostatic, and hydrogen bonding) are used to develop advanced packaging systems that possess antimicrobial activities (Morris et al. 2017). On the other hand, for nanocomposite production, polymers such as ethylene–vinyl acetate copolymer, polyolefins, nylons, polyethylene terephthalate, polystyrene, and polyamides can be used because polymer matrices control the release of active components and regulate the function of nanocomposites (Nie et al. 2020). The studies conducted for the potential application in food and agriculture fields of nanoparticles are given in Table 7.

As a result, the application of NPs is vast in the food industry, spanning from ingredients to packaging, and product analysis. Despite its potential, the interaction of NPs with food systems can cause health concerns for humans and animals. Nanoformulated products can be toxic to plants and animals, yet no standard regulatory laws exist for their use in the food and agriculture sectors. Thus, effective guidelines and policies should be legislated as crucial for the safe use of NPs in the food industry (Nie et al. 2020). In the European Union, “Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)” conducts risk assessments for nanotechnology. EU regulations highlight that nanotechnology-based food ingredients should undergo safety evaluations before authorization for consumption (Tinkle et al. 2014; Nie et al. 2020).

4. Challenges and future prospects

NP synthesis through green methods, especially by using beneficial microorganisms, has garnered significant attention because of its eco-friendly nature and potential applications in various fields such as medicine, agriculture, food, and environmental remediation. However, this area of research is not without its challenges, and addressing these is significant for future advancements and applications.

The reproducibility and scalability of NP synthesis using microorganisms pose important challenges. Variability in biological systems can lead to inconsistent NP characteristics, affecting their performance and application. Besides, scaling up from laboratory to industrial production while maintaining the NP's quality and characteristics is complex and requires standardization of processes (Gomez-Zavaglia et al. 2022). In this regard, genetic engineering could play a vital role in producing microorganisms with enhanced reproducibility, thus overcoming some of the scalability challenges.

On the other hand, controlling the shape, size, and crystallinity of NPs synthesized by microorganisms is also challenging. Contrary to chemical synthesis, where conditions can be finely tuned, biological synthesis is influenced by many factors such as the microorganism's metabolic state, growth conditions, and the presence of other biomolecules. To address this, advanced bioreactors with precise control over parameters like pH, temperature, and nutrient availability could enhance NP consistency and quality. Achieving uniformity in NP properties is important for their usages, particularly in fields, such as drug delivery and food (Gomez-Zavaglia et al. 2022; Shanmugam et al. 2023; Osman et al. 2024).

The other one, understanding the exact mechanisms by which microorganisms synthesize NPs is still incomplete. Different microorganisms use varied biochemical pathways for NP synthesis, and these pathways can be both intracellular and extracellular. Comprehensive knowledge of these mechanisms is important for optimizing and controlling the synthesis process (Shanmugam et al. 2023). Future work should focus on identifying and controlling these pathways to improve NP synthesis. The type of microorganisms can also affect the characteristics of nanoparticles synthesized by green technology. Some properties of ZnO NPs synthesized by different beneficial microorganisms (*Lactobacillus* sp., *L. plantarum* VITES07, and *Saccharomyces cerevisiae*) are presented and compared in Figure 5. While the shape of all ZnO NPs synthesized by different beneficial microorganisms was observed as spherical by SEM analyses, the sizes of nanoparticles were measured as 32 nm, 7-19 nm, and 13-20 nm for *Lactobacillus* sp., *L. plantarum* VITES07 and *S. cerevisiae*, respectively. The average crystalline sizes of ZnO NPs were also calculated by XRD peaks using Scherrer’s equation as 7 nm, 18.6 nm, and 13.58 nm, respectively. FTIR results showed the ZnO stretching vibrations patterns were correlated with 528 cm^{-1}, 641 cm^{-1}, and 570.2 cm^{-1} for NPs synthesized by *L. plantarum* VITES07, *Lactobacillus* sp. and *S. cerevisiae*, respectively. Ensuring the biocompatibility and non-toxicity of biosynthesized NPs is vital, especially for medical and food applications. The presence of residual biomolecules from the synthesis process can impact the toxicity profile of NPs. Detailed toxicological studies are required to ensure safety and efficacy, which can be time-consuming and resource-intensive (Kışla et al. 2023).

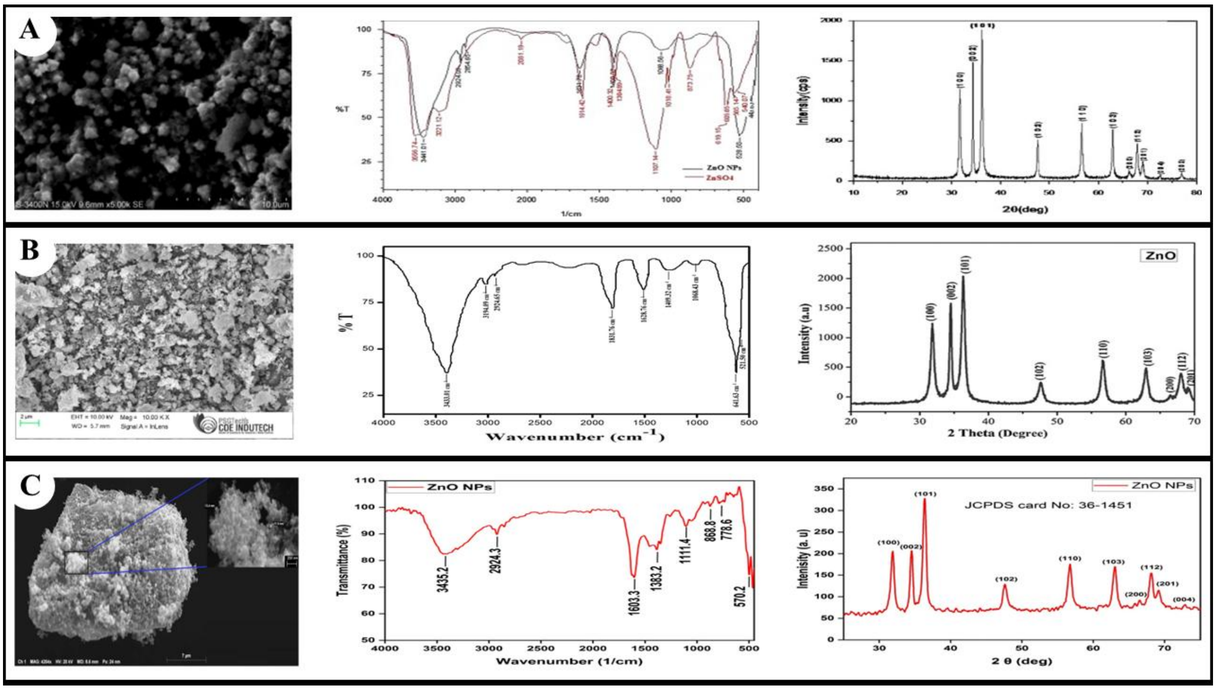

Figure 5- Comparison of the properties of ZnO NPs synthesized by different beneficial microorganisms including *Lactiplantibacillus plantarum* VITES07 (A) (Selvarajan et al. 2013), *Lactobacillus* sp. (B) (Suba et al. 2021), and *Saccharomyces cerevisiae* (C) (El-Khawaga et al. 2023)

While green synthesis is environmentally friendly, its economic viability is a concern factor. The costs associated with cultivating microorganisms, optimizing growth conditions, and harvesting NPs can be high. Besides, the extraction and purification processes should be efficient to make the overall process cost-effective compared to conventional methods (Alsaiari et al. 2023; Osman et al. 2024). Nevertheless, advances in genetic engineering offer promising solutions to improve the efficiency and control of NP synthesis. By manipulating the genetic pathways involved in metal ion reduction and NP formation, microorganisms can be engineered to synthesize NPs with desired characteristics. This approach could increase the reproducibility and scalability of biosynthesis processes. Also, detailed studies on optimizing the growth conditions of microorganisms can lead to more consistent and controllable NP synthesis. Factors such as pH, temperature, nutrient availability, and the presence of specific inducers need to be finely tuned to maximize NP yield and quality. Developing bioreactors with precise control over these parameters could facilitate large-scale production. Combining biological and chemical synthesis methods could overcome some of the limitations of pure biological synthesis (Alsaiari et al. 2023).

Post-synthesis functionalization and surface modification of NPs can also improve their biocompatibility, stability, and functionality. Techniques such as coating NPs with peptides or biopolymers etc. can improve their dispersibility in different media and target-specific applications. This is particularly important for medical applications where targeted drug delivery is significant (Gavas et al. 2021; Kışla et al. 2023). Conducting application-specific research to tailor NPs for particular uses can accelerate their adoption in various fields. For example, in agriculture, NPs can be designed for slow-release fertilizers or pesticides, while in medicine, they can be used for specific drug delivery systems or imaging agents (Liu et al. 2021; Sivarethinamohan & Sujatha 2021). So, collaborative research across disciplines can lead to innovative solutions and practical applications of synthesized NPs.

A comprehensive assessment of the environmental impact of NP synthesis and application is necessary. Despite green synthesis being eco-friendly compared to traditional methods, the long-term effects of NPs in the environment need to be understood. For this scope, in addition to the increase in the synthesis of NPs with different types, shapes, and characteristics, the scientific community has increasingly focused on examining their environmental impact, as well as their effects on living things (Martínez et al. 2021). Multiple pathways exist for NPs to enter the environment, these sources can be categorized into point sources (such as factories, landfills, and wastewater treatment plants) and non-point sources (materials containing NPs). Besides, NPs are directly introduced into the environment through applications such as remediation technologies, causing their aggregation and subsequent infiltration into soil, groundwater, and sediments. This has resulted in a novel research domain described as "Nanoecotoxicology" (Bimová et al. 2021). The intrinsic toxicity of NPs can be related to their propensity to reach and accumulate in air, soil, and water, with their effect directly proportional to their degree of accumulation. The risks of NPs are affected by various processes that impact their release mechanisms (Martínez et al. 2021). The principal mechanism underlying the potential toxicity of NPs includes their property to induce peroxidation of membrane lipids and oxidative stress, which subsequently causes the generation of ROS and resultant damage to cellular structures (Czyżowska & Barbasz 2022). While the application of NPs offers numerous advantages across various industries, it is imperative to consider and address their potential negative effects on the environment. Hence, toxicology studies of NP synthesized on negative effects not only on humans but also on the environment should be thoroughly studied. The interaction of these NPs with crucial nutrients, especially for plants is also a compelling area of research. So, a detailed assessment of all factors such as application method, exposure time, and environmental factors, as well as NP's shape, size, and concentration is required (Bundschuh et al. 2016; Selmani et al. 2020).

As a result, while the green synthesis of NPs using beneficial microorganisms holds great promise, several challenges must be addressed to fully realize its potential. Future research should focus on improving reproducibility, scalability, and understanding the underlying mechanisms of NP synthesis, alongside advancements in genetic engineering, hybrid synthesis, and environmental safety. Collaborative efforts between microbiologists, engineers, chemists, and material scientists will be crucial to overcome these challenges and enable the widespread application of green-synthesized NPs.

5. Conclusions

The green synthesis of NPs offers a sustainable and environmentally friendly alternative to traditional chemical and physical methods. Beneficial microorganisms have also attracted special interest in the green synthesis of NPs due to their non-pathogenic properties of them, high production of various enzymes, as well as GRAS status that allows their use in various food and nutraceutical products. In this review, NP synthesis by beneficial microorganisms as green synthesis has been highlighted, and the potential application fields of NPs summarising their efficiency have been described. The green synthesis approach leverages the natural metabolic processes of microorganisms to produce NPs with unique properties suitable for various applications. Different NPs such as Ag, Se, Pd, Sb_2O_3, ZnO, and TiO_2 are synthesized by beneficial microorganisms, and these NPs have various characteristics in terms of size (20-300 nm), morphology (spherical or hexagonal), as well as characteristic properties such as antimicrobial, antioxidant, antiproliferative, anticancer, and antidiabetic activity, as presented in this review. Besides, microbial NPs have potential use in various fields as antimicrobial, anticancer, nanocarrier, and nanofertilizer, and can also play an important role in enhancing food quality and safety through developed processing and/or packaging, and long-term storage techniques. Despite the evident potential and diverse applications, significant challenges remain in ensuring reproducibility, controlling NP characteristics, understanding synthesis mechanisms, and ensuring economic viability. For this scope, the toxicity effect of NP synthesis and applications on living things and the environment should be comprehensively assessed. In conclusion, while microbial synthesis of NP presents several challenges, the future prospects are bright. Future research focused on application-specific development and comprehensive environmental impact assessments will be crucial in harnessing the full potential of this green synthesis method. As the field progresses, the integration of interdisciplinary research and innovative technological approaches will likely lead to significant advancements, making synthesized NP a cornerstone in various industries, especially in food, agriculture, and medical applications.

Conflicts of Interest

The author declares that there is no conflict of interest to disclose.

Funding

The author declares that the study received no funding.

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