Microorganisms as Nano-factories for the Synthesis of Metal Nanoparticles

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Abstract: Nanoparticles applications have revolutionized different areas of the research. These include medicine, surgery, drug delivery, wastewater treatment, agriculture, cancer therapy, etc. The use of nanoparticles is increasing day by day due to their promising characteristics. With the excessive use of the nanoparticles, their accumulation in the organisms and different environments has been reported. A very high increase in the accumulation and toxicity of nanoparticles has been reported in the last decade. Therefore, the nanoparticle research has now been shifted to find new techniques and methods to minimize the toxic effects of nanoparticles. In this context, the requirement of a safe design approach and the generation of fewer toxic nanoparticles are required. One of the eco-friendly approaches for safer nanoparticles synthesis is the use of living organisms for nanoparticles production. Microbes especially, bacteria, fungi, and yeasts, are considered safe, secure, and efficient systems for nanoparticle biosynthesis. This review is an attempt to understand the potential of microbes for the biogenic nanoparticles synthesis.

Keywords: Nano-factories, biosynthesized nanoparticles, microorganisms, biogenic nanoparticles, toxicity reduction, eco-friendly.

1. INTRODUCTION

Nanoparticles have been used as promising candidates in diverse areas, including medicine. There are various strategies for the synthesis of nanoparticles. The most common strategy to produce nanoparticles involves the utilization of toxic organic solvents and reducing agents. These reagents are used to prevent agglomeration and defects in the nanoparticles [1]. The use of chemical methods is hazardous due to the use of toxic chemicals, such as hydrazine, dimethyl sulfoxide, acetonitrile, methanol, acetone, toluene, and potassium bitartrate [2, 3]. These chemicals are responsible for genotoxicity, carcinogenicity, and cytotoxicity [4, 5]. The use of these reagents is not environmentally-friendly. Therefore, various green matrices, such as DNA, bacteria, peptide, amino acid, protein, natural products, and polysaccharide were used for biomimetic synthesis [6-12]. With the increasing toxicity imposed by the nanoparticles on the living organisms. The necessity for the discovery of nanoparticle toxicity reduction methods is observed. In this connection, few strategies have been proposed. One of them is the use of eco-friendly approaches, such as biogenic nanoparticles. The use of biological organisms for the production of nanoparticles has emerged as a promising solution to the traditional nanoparticles production methods [13]. Fig. (1) presents different microorganisms, which can be used for the biogenic nanoparticles production.

2. BIOGENIC NANOPARTICLES

Biogenic nanoparticles are produced using living organisms. These nanoparticles are produced from bacteria, fungi, yeast, etc. They are alternative to the toxic nanoparticles, which are artificially synthesized. Biogenic nanoparticles have greater acceptance in the medical applications because of their advantages over the chemically produced nanoparticles [14]. Fig. (2) presents various advantages offered by biogenic nanoparticles. They offer biocompatibility, which gives them added stability and improved interaction with the pathogens involved [15]. Other advantages of using biogenic nanoparticles include low cost, reduced toxicity, and high efficiency [16]. Table 1 represents a few methods used in the biogenic nanoparticles synthesis along with their advantages.
Table 1. Methods for the production of nanoparticles using microorganisms.

| Nanoparticle Synthesis Method | Advantages | Disadvantages | References |
|------------------------------|------------|---------------|------------|
| Bioreduction: Microorganisms are used to reduce metal ions into biologically stable form | Nanostructures are inert and stable in nature. | - | (Deplanche, Caldelari, Mikheenko, Sargent, & Macaskie, 2010; Pantidos & Horsfall, 2014) [143, 144] |
| Biosorption: Metal ions bind to the organism cell wall | Cell wall and nanoparticles interaction cause the formation of stable nanoparticles | - | (Pantidos & Horsfall, 2014; Yong, Rowson, Farr, Harris, & Macaskie, 2002) [144, 145] |
| Nanoparticles synthesis using bacteria | Bacteria are abundant for various applications. Bacteria can adapt to extreme conditions. | Safety risk | (Pantidos & Horsfall, 2014) [144] |
| Nanoparticles synthesis using fungi | Synthesized particles are easy to scale up and downstream processing. Fungi secrete more amount of proteins | Produced nanoparticles vary in size | Safety risk | (Pantidos & Horsfall, 2014) [144] |

Fig. (1). Microorganisms used to produce biosynthesized nanoparticles. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

Fig. (2). Advantages of using biosynthesized nanoparticles.
and disadvantages. In a study, the importance of biogenic silver nanoparticles was demonstrated. As compared to the synthetic silver nanoparticles, the silver nanoparticles produced from *Aspergillus tubingensis* were less harmful to the soil microbiota [17]. In the study, varying concentrations of *Aspergillus tubingensis* produced silver nanoparticles were used on rice seeds, soil microorganisms, and zebrafish. The study demonstrated better performance of the *Aspergillus tubingensis* produced biogenic silver nanoparticles over synthetic nanoparticles. A recent study demonstrated that magnetic nanoparticles could be synthesized in human stem cells from the nano-degradation products [18]. The study was performed by internalization in the mesenchyme stem cells. The nanoparticles were degraded during chondrogenesis, and stem cells were remagnetized. This remagnetization was the direct demonstration of magnetic nanoparticle's biosynthesis.

3. MICROBIAL SYNTHESIS OF NANOPARTICLES

Microorganisms, such as actinomycetes, bacteria, fungi, and yeast received attention since the past decade to produce nanoparticles [19]. Microorganisms are known to precipitate the nanoparticles by their metabolic activities [20]. Microorganism produces inorganic nanoparticles with immense applications in the biomedical fields [21]. Different microorganisms have been used for the synthesis of silver, gold, copper, and zinc nanoparticles [22-25]. Fig. (3) presents various applications involved in the use of biogenic nanoparticles. Table 2 represents microorganisms, along with the types of nanoparticles produced.

### Table 2. Types of organisms and nanoparticles.

| Bacteria                        | Types of Nanoparticles | Size of Nanoparticles | References                                      |
|---------------------------------|------------------------|-----------------------|------------------------------------------------|
| *M. psychrotolerans*53          | Silver nanoparticles   | -                     | (Ramanathan *et al.*, 2011) [91]                |
| *E. coli*                       | Silver                 | -                     | (Huang *et al.*, 2015) [140]                    |
| *Pseudomonas aerogenosa* JP2    | Silver                 | -                     | (Ali *et al.*, 2017) [146]                      |
| *Pseudomonas aerogenosa* JP1    | Silver                 | -                     | (Ali *et al.*, 2016) [147]                      |
| *Verticillium sp.*              | Magnetite              | -                     | (Bharde *et al.*, 2006) [148]                   |
| *Geobacillus* sp. strain ID17   | Gold                   | 5-50 nm               | (Correa-Llantén, Muñoz-Ibacache, Castro, Muñoz, & Blamey, 2013) [150] |
| *E. coli* DH5α                  | Gold                   | 8-25 nm               | (Du, Jiang, Liu, & Wang, 2007) [152]            |
| *Tetraothiobacter*              | Selenium               | -                     | (Hunter & Manter, 2008) [157]                   |
| *Bacillus* licheniformis        | Gold                   | 10-100 nm             | (Kalishwaralal, Deepak, Ram Kumar Pandian, & Gurunathan, 2009) [158] |
| *Brevibacterium* casei          | Gold                   | 10-50 nm              | (Kalishwaralal *et al.*, 2010) [159]            |
| *Lactobacillus* casei           | Gold                   | 7-56 nm               | (Kikuchi *et al.*, 2016) [159]                  |
| *Streptomyces* clavuligerus     | Gold                   | 8.2 nm                | (C. G. Kumar, Poornachandra, & Chandrasekhar, 2015) [161] |

Fig. (3). Applications of the biosynthesized nanoparticles.
| Bacteria                      | Types of Nanoparticles | Size of Nanoparticles | References                                                                 |
|-------------------------------|------------------------|-----------------------|---------------------------------------------------------------------------|
| *Shewanella oneidensis*       | Silver                 | -                     | (Law, Ansari, Livens, Renshaw, & Lloyd, 2008) [163]                      |
| *Bacillus stearothermophilus* | Gold                   | 5-30 nm               | (Luo, Liu, Xia, Xu, & Xie, 2014) [164]                                   |
| *D. desulfuricans* ATCC 29577 | Pelladium              | -                     | (Mabbett, Yong, Farr, & Macaskie, 2004) [164]                            |
| *Klebsiella pneumoniae*       | Gold                   | 35-65 nm              | (Malarkodi et al., 2013) [166]                                           |
| *Lactobacillus kimchicus* DCY51 | Gold                  | 5-30 nm               | (Markus et al., 2016) [167]                                              |
| *Geobacillus stearothermophilus* | Gold               | 12-14 nm            | (Mohammed Fayaz, Girilal, Rahman, Venkatesan, & Kalaichelvan, 2011) [171] |
| *Stenotrophomonas maltophilia* | Gold                 | 40 nm                 | (Nangia, Wangoo, Goyal, Shekhawat, & Suri, 2009) [172]                   |
| *S. maltophilia*              | Silver                 | -                     | (Oves et al., 2013) [174]                                                |
| *Rhodopseudomonas capsulata*  | Gold                   | 10-20 nm              | (Parab, Jung, Lee, & Park, 2010) [175]                                   |
| *Bacillus Subtilis*           | Gold                   | 10-15 nm              | (Satyanarayana Reddy et al., 2010) [182]                                 |
| *Enterobacter cloacae*        | Silver                 | -                     | (Shahverdi, Minaeian, Shahverdi, Jamalifar, & Nohi, 2007) [185]          |
| *E. coli K12*                 | Gold                   | 50 nm                 | (Srivastava, Yamada, Ongino, & Konno, 2013) [188]                        |
| *Shewanella oneidensis*       | Gold                   | 2-50 nm               | (Suresh et al., 2011) [188]                                              |
| *Acinetobacter sp. SW 30*     | Gold                   | 39 nm                 | (Wadhwani, Shedbalkar, Singh, Karve, & Chopade, 2014) [191]              |

| Fungi                    | Type of Nanoparticles | Size of Nanoparticles | References                                                                 |
|--------------------------|-----------------------|-----------------------|---------------------------------------------------------------------------|
| *Penicillium brevicompactum* | Gold                 | 10-60 nm              | (Mishra et al., 2011) [83]                                               |
| *Rhizopus oryzae*        | Gold                  | 16-25 nm              | (Das, Dickinson, Lafir, Brougham, & Marsili, 2012) [151]                 |
| *Alternaria sp.*         | Gold                  | 7-93 nm               | (Dhanasekar, Rahul, Narayanan, Raman, & Sakthivel, 2015) [153]           |
| *Helmintosporum solani*  | Gold                  | 2-70 nm               | (S. A. Kumar, Peter, & Nadeau, 2008) [162]                               |
| *Penicillium citrinum*   | Gold                  | 60-80 nm              | (Manjunath, Joshi, & Raju, 2017) [167]                                   |
| *Penicillium rugosum*    | Gold                  | 20-80 nm              | (Mishra, Tripathy, & Yun, 2012) [170]                                   |
| *Cylindrocladium floridanum* | Gold                | 5-35 nm               | (Narayanan & Sakthivel, 2013) [172]                                     |
| *Volvariella volvacea*   | Gold                  | 20-150 nm             | (Philip, 2009) [177]                                                    |
| *Neurospora crassa*      | Gold                  | 32 (3-100) nm         | (Quester, Borja, Nestor, Lopez, & Longoria, 2013) [179]                  |
| *Alternaria alternate*   | Gold                  | 12 ± 5 nm             | (Sarkar, Ray, Chattopadhyay, Laskar, & Acharya, 2012) [181]              |

(Table 2) contd....
3.1. Nanoparticles Biosynthesis in Bacteria

Bacteria are a popular target for nanoparticles production due to their easier genetic manipulation and higher growth rates [26]. Recently, bacterium Desulfovibrio vulgaris has been used to produce nanoparticles useful in pharmaceutical compounds removal [27]. A gram-negative bacteria Acinetobacter calcoaceticus produced monodispersed cuboidal nanoparticles [28]. Another bacteria Shewanella algaë stored the nanoparticles in periplasmic space [29]. Filamentous cyanobacteria such as Calothrix and Plectonema boryanum were known to produce nanoparticles with the size 3.2 and 5, respectively [29, 30]. In a very recent report, biogenic copper nanoparticles were synthesized from Escherichia sp. for textile effluent treatment and azo dye degradation [31].

In a recent study, copper nanoparticles synthesized from Escherichia sp. were used for the treatment of textile effluents and azo dye photocatalysis [31].

3.2. Nanoparticles Biosynthesis in Fungi

Nanoparticle biosynthesis from fungi is another simple method which has been recently used. Fungi have enormous potential for nanoparticle production as compared to the bacteria. They have higher productivity and higher metal ions tolerance [13]. The biomass treatments and downstream processing are relatively straightforward in fungi when compared to bacteria. Also, they have metal ions bioaccumulation capacity, which results in cost-effective and efficient nanoparticle production. In this context, fungi Cladosporium oxysporum was used for the production of gold nanoparti-
cles. The study investigated the effect of salt concentration, pH, and reaction time on yield and particle size [32]. The biosynthesized gold nanoparticles exhibited efficient catalytic activity in textile dye degradation. In another study, the effect of incubation time and reaction temperature were observed for the production of extracellular gold nanoparticles in the culture filtrate of Trichoderma viride and Hypocrea lixi [33]. White rot fungus, Schizophyllum radiatum isolated from the forest, had the potential to produce silver nanoparticles with the size range 10-40 nm [34]. The produced silver nanoparticles exhibited significant antibacterial activity against a variety of bacterial strains. Fungi Neurospora crassa produced nanoparticles in various shapes such as round, crystalline and quasi-spherical [35]. Another fungus Fusarium oxysporum isolated from the shape direction of the gold nanoparticles. The study evidenced the gold reduction in the thylakoids. Tetraselmis suecica produced spherical gold nanoparticles in the range 51-120 nm [69]. Chlorella pyrenoidosa produced spherical and icosahedral gold nanoparticles in the range of 25-30 nm using the NADH-dependent enzyme [70]. Tetraselmis kochiihensis produced triangular and spherical gold nanoparticles in the range of 5-35 nm using reducing enzymes in the cytoplasmic membrane and cell wall [71].

Algae can produce secondary metabolites and proteins, which can be used to produce potential metal nanoparticles [72-75]. The preparation of algae mediated nanoparticles synthesis involves algal extract preparation. The extract is then mixed with the metal precursor solution [76]. This procedure can be used to form stable nanoparticles in terms of different shapes and sizes [58, 76, 77]. Algae mediated nanoparticle synthesis can be intracellular [78] and extracellular [58, 79]. Spirulina subsalsa and Lyngbya majuscula were used for the production of gold nanoparticles [80]. Spherical nanoparticles were produced from the alga Prasiola crispa [81].

Discussed here are a few very recent studies where algae were used to synthesize nanoparticles. In a study, silver nanoparticle was synthesized using algae. The study investigated the effect of silver nanoparticle formation on the life cycle of algae [82]. Silver nanoparticles were synthesized using green algae Botryococcus braunii, red algae Portieria hornemannii and Gelidium corneum [83-85]. A comparison was made for the two red algae for silver nanoparticles biosynthesis [86]. A macro algae polysaccharide was used for the silver nanoparticle's green synthesis [87]. An algae obtained from the Mediterranean sea was used for the biogenic synthesis of iron oxide nanoparticles [88]. A brown algae Cystoseira baccata was used for the gold nanoparticle's green synthesis [72].

### 3.3. Nanoparticles Biosynthesis in Yeast

Yeast species have also been investigated for nanoparticle biosynthesis. The reason for using yeast as the nanofactories is that they possess the inherent potential to absorb and accumulate very high concentrations of toxic metal ions [38]. There are various reports of nanoparticle biosynthesis from yeast. These include Rhodospiridium diobovatum biosynthesized (lead sulfide nanoparticles, 2-5 nm) [39], Yarrowia lipolytica NCYC789 (silver nanoparticles, 15 nm) [40], Candida utilis NCIM 3469 (silver nanoparticles, 20-80 nm) [41], Cryptococcus laurentii (silver nanoparticles, 35 nm) [42], Saccharomyces cerevisiae (silver nanoparticles, 2-20 nm) [43], Magnusiomyces ingen LHF1) [44], Pichia pastoris (silver nanoparticles, 70-180 nm) [45], Candida lusitaniae (silver and silver chloride, 2-10 nm) [46], Rhodotorula glutinis, Cryptococcus laurentii (silver nanoparticles, 15-35 nm) [47], Saccharomyces cerevisiae (gold nanoparticles) [48], Candida albicans ATCC 10231 (silver nanoparticles, 10-20 nm) [49], Pichia kudriavzevii (zinc oxide nanoparticles, 10-61 nm) [50], Rhodotorula mucilaginosa (silver nanoparticles, 11 nm) [51], Magnusiomyces ingen LHF1 (gold nanoparticles, 20.3-28.3) [52], Rhodotorula glutinis (silver nanoparticles, 15 nm) [53], Saccharomyces cerevisiae (palladium nanoparticles, 32 nm) [54] and Candida glabrata (2-15 nm) [55].

### 3.4. Nanoparticle Biosynthesis in Algae

Algae are considered as promising candidate research due to their different properties. One of these properties is their ability to accumulate heavy metal ions [56, 57]. After accumulation, metal ions can be transformed into useful materials [58]. Various properties mark the algae as nanofactories. These properties include lower doubling time, simple cell disruptions, quick harvesting, easy scalability at a high scale and low-cost production [59-63]. Various micro, as well as macroalgae, has been known to produce nanoparticles [64-66].

In microalgae, various strains have been used for the production of nanoparticles. Chlorella vulgaris produced up to 2 µm and 60 nm gold nanoparticles [67]. In this strain, a protein of 28kDa was identified, which was responsible for the shape direction of the gold nanoparticles. Klebsormidium Flaccidum produced gold nanoparticles in the range of 10-20 nm with the Sol-gel methods [68]. The study evidenced the gold reduction in the thylakoids. Tetraselmis suecica produced spherical gold nanoparticles in the range 51-120 nm [69]. Chlorella pyrenoidosa produced spherical and icosahedral gold nanoparticles in the range of 25-30 nm using the NADH-dependent enzyme [70]. Tetraselmis kochiihensis produced triangular and spherical gold nanoparticles in the range of 5-35 nm using reducing enzymes in the cytoplasmic membrane and cell wall [71].

A variety of factors are responsible for the reduction of metals ions to biosynthesize the nanoparticles in the microorganisms. One of them is the presence of an organic functional group on the cell wall, which is responsible for the induction of biomineralization of the metal ions. Another critical factor is the environmental conditions, which include pH, temperature, medium composition, and metal salt concentration [89]. These environmental conditions are known to affect nanoparticle morphology, size, and composition [90]. The efficiency of nanoparticle biosynthesis can be enhanced by the optimization of the factors. In such an effort, growth kinetics was optimized for the biosynthesis of silver nanoparticles using Morganella psychrotolerans to observe the effects on the nanoparticle’s morphology [91]. In the study, the temperature played an important role where spherical nanoparticles were produced at 20°C, mixed spherical, hexagonal and triangular nanoparticles at 25°C, mixed nanoparticles and spherical at 15°C and significant enhancement of nanoparticles number at 4°C. The biosynthesis of silver nanoparticles produced by Arthrobacter sp. demonstrated that factors such as pH, temperature, and metal ions concentrations could modulate nanoparticle synthesis [92]. The study
demonstrated that a reduction in the silver nitrate concentration caused the synthesis of face-centered cubic nanoparticles, and increased silver nitrate concentration resulted in the aggregation of silver nanoparticles. The study concluded that metal ion concentration and medium pH could have a direct influence on nanoparticle synthesis.

In a study conducted for the biosynthesis of gold and silver nanoparticles demonstrated that low molecular weight secretory proteins which were present in the supernatant, which was responsible for nanoparticles synthesis [93]. Several studies reported the nanoparticle biosynthesis via bacterial extracellular polymeric substances (EPS) [94-96]. These substances act as effective capping and bio-reductant agents. In a similar study, EPS secreted from a marine isolate was used to produce silver nanoparticles [94].

4.1. Mechanisms of Nanoparticles Biosynthesis in Bacteria

In bacteria, nanoparticles can be produced either extracellularly or intracellularly. A study reported the deposition of gold nanoparticles first time on the cell wall of the bacterium Bacillus subtilis extracellularly [97]. Intracellular accumulation of silver nanoparticles was reported in a silver resistant Pseudomonas stutzeri AG259 [98]. Another Pseudomonas strain Pseudomonas aeruginosa had to synthesize a variety of metal nanoparticles [89]. Silver nanoparticles were produced by Bacillus sp. CS11 (spherical, 42-92 nm) [99], Bacillus brevis NCIM 2533 (spherical, 41-68 nm) [100], Bacillus cereus (spherical 20-40 nm) [101], Alteromonas macleodi (spherical, 70 nm) [94], Stenotrophomonas GSG2 (triangular, circular and hexagonal, 40-60 nm) [93], Alcaligenes faealci (spherical, 30-50 nm) [102], Deinococcus radiodurans (spherical, 4-50 nm) [103], Ochrobactrum rhizosphaerae (spherical, 10 nm) [96], Pseudomonas aeruginosa (spherical and triangular 35-60 nm), Pseudomonas aeruginosa JP-11 (spherical, 20-40 nm) [94], Escherichia coli, Klebsiella pneumoniae and Pseudomonas jessinii (spherical, 50-100 nm) [104]. Copper nanoparticles, spherical in shape, and size range 5-30 nm were produced in Kocuria flava [105]. Cadmium sulfide nanoparticles, spherical with a size range of 20-40 nm were produced in Pseudomonas aeruginosa JP-11 [95]. Palladium and platinum nanoparticles were produced by Shewanella loihica PV-4, which were spherical with a size range of 2-7 nm [106]. Tellurium nanoparticles were produced by Ochrobactrum sp. MPV1 [107]. Gold nanoparticles spherical in shape with a size range of 20-25 nm were produced by Bacillus subtilis [108].

A bacterium Bacillus cereus was isolated from a site contaminated with heavy metals. The bacterium synthesized extracellular silver nanoparticles with surface plasmon resonance properties [99]. These properties could be used in various applications. In an experiment, radiation-resistant strain Deinococcus radiodurans biosynthesized extracellular silver nanoparticles by silver chloride solution reduction [103]. The biosynthesized silver nanoparticles demonstrated antibacterial, anti-biofilm and anti-cancerous activities. In a study, palladium and platinum nanoparticles were synthesized, which demonstrated methyl orange dye decomposition [106]. In a different study, Ochrobactrum sp. produced tellurium nanoparticles, which demonstrated the potential of this bacterium for the conversion of tellurite oxoanions to the useful nanoparticles [107]. The toxic gold ions were converted to the gold nanoparticles by Bacillus subtilis [108]. The produced gold nanoparticles were used as a biocatalyst for methylene blue degradation and can be used for degradation of other dyes toxic to the environment. Silver nanoparticles produced by Bacillus brevis demonstrated antibacterial activities against drug-resistant strains of Staphylococcus Aureus and Salmonella typhi [109].

4.2. Mechanisms of Nanoparticles Biosynthesis in Actinomycetes

Actinomycetes are a class of microorganisms that are used to produce secondary metabolites [109]. They have been considered to produce nanoparticles due to their high protein content and bioactive compounds. The extracellular reduction is the major pathway involved in the nanoparticle production in actinomycetes. Silver nanoparticles were synthesized with Rhodococcus NCIM 2891 (spherical, 10 nm) [110], Rhodococcus sp. NCIM 2891 (spherical, 10-15 nm) [111], Streptomyces sp. LK3 (spherical, 5 nm) [112], Streptacidiphilus Durhamensis (spherical, 8-48 nm) [113], Streptomyces rochei MHM13 (spherical, 22-85 nm) [114], Streptomyces parvulus (spherical, 1-40 nm) [115] and Streptomyces xinghaiensis OF1(spherical, 5-20 nm) [116]. Gold nanoparticles were produced by Streptomyces griseoruber (spherical, 5-50 nm) [117]. Copper nanoparticles were produced by Streptomyces capilli spiralis Ca-1 (spherical, 3.6-59 nm) [118].

4.3. Mechanisms of Nanoparticles Biosynthesis in Yeast Cells

Yeast cells adapt themselves in the metal toxicity conditions by detoxification mechanisms such as chelation, precipitation and intracellular sequestration. In a study, Yarrowia lipolytica yeast cells were used for the biosynthesis of silver nanoparticles. The study demonstrated that brown pigment in the yeast cells was responsible for the biomineralization of metal ions and pigment derived nanoparticles exhibited antibiofilm activity against pathogen Salmonella paratyphi [40]. Extracellular eco-friendly silver nanoparticles were synthesized by Candida utilis NCIM 3469 [41]. The produced nanoparticles were circular, with a size range of 20-80 nm and had antibacterial activity against pathogenic strains such as Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. A genetically modified Pichia pastoris was used for silver nanoparticles biosynthesis [45]. The yeast overexpressed cytochrome b5 reductase enzyme, which was responsible for metal ions reduction to the nanoparticles. A yeast strain, Candida Lusitania isolated from termite gut, produced silver nanoparticles in the size range 2-10 nm [46]. The biosynthesized nanoparticles exhibited anti-proliferative activity to Klebsiella pneumoniae and S. aureus. In another study, Saccharomyces cerevisiae was used for the biosynthesis of palladium nanoparticles, which exhibited photocatalytic textile azo dye degradation [54].

4.4. Mechanisms for Intracellular Nanoparticle Biosynthesis

In the mechanism of intracellular biomineralization of silver ions, it was considered that the enzymes present on the cell wall of the bacteria were responsible for the production
of silver nuclei. In a study, silver nanoparticles were produced by the reduction of silver ions by \textit{Streptomyces} sp. LK-3 [112]. The study reported that the extracellular production of stable silver nanoparticles was conducted by NADH-reductase by an electron transfer reaction. The produced nanoparticles exhibited strong antiparasitic and acaricidal activities. In a recent study, \textit{Streptacidiphilus durhamensis} was used for the synthesis of silver nanoparticles [113]. The produced nanoparticles demonstrated antimicrobial activity against \textit{Proteus mirabilis}, \textit{Staphylococcus aureus} and \textit{Pseudomonas aeruginosa}. In general, the nanoparticles synthesized from the microorganisms have higher antimicrobial activities due to the stabilization and capping of the nanoparticles as compared to the traditional nanoparticles synthesized.

### 4.5. Mechanisms for Extracellular Nanoparticle Biosynthesis

Most of the studies reported have demonstrated the extracellular secretion of the nanomaterials. The advantages of extracellular nanoparticle synthesis are that it is devoid of impurities such as intracellular proteins. Moreover, treatment of detergent is not involved, and ultrasound is not required. Understanding the mechanism of nanoparticle biosynthesis in fungi is indispensable for various applications. In this context, the effect of temperature, pH and isolate selection on nanoparticle morphology was observed in the nanoparticle production using \textit{Fusarium oxysporum} [119]. Aluminum oxide nanoparticles were synthesized using \textit{Colletotrichum} sp., and the synthesized nanoparticles were functionalized by essential oils [120]. The study results demonstrated that functionalized oil could be used as an antimicrobial agent for food-borne pathogens. Recently, antimicrobial and anticancer nanoparticles have been synthesized using edible mushroom \textit{Pleurotus ostreatus} and two fungi \textit{Scopulariopsis brumptii} and \textit{Penicillium citreonigrum} [121].

### 5. BIogeneN NANOPARTICLES CYTOTOXICITY

There are various routes through which organisms come in contact with the nanoparticles. Humans can be exposed to nanoparticles through inhalation, skin contact, and gastrointestinal absorption [122]. After entering the human body, nanoparticles can be engulfed by the macrophages, which may lead to the development of inflammation [123, 124]. On ingestion, it reaches the liver and accumulates there [125]. Nanoparticle accumulation results in the glutathione level alteration increased reactive oxygen species (ROS) and mitochondrial potential reduction [126]. Nanoparticles are also known to induce oxidative stress in the respiratory system [127].

Studies have reported that silver nanoparticles can disturb the plasma membrane integrity, which leads to leakage [128]. The normal function of the mitochondrial membrane can be impaired with the nanoparticle interaction [129]. In a study, nanoparticles caused the mitochondrial membrane lipids peroxidation after accumulation around the mitochondria [130]. Reactive Oxygen Production mediated apoptosis was observed in the cells exposed to metal nanoparticles [131]. DNA damage is caused after nanoparticles enter the nucleus [132]. Nanoparticles exposure can disturb regular cell metabolism leading to apoptosis and cell death [133]. Apoptosis mediated cell death is governed by combined molecular mechanisms, such as DNA fragmentation and caspase pathway [134].

All the toxicity, as mentioned above, were reported for artificially synthesized nanoparticles. Biosynthesized nanoparticles also cause some toxicity. In a bacteria mediated nanoparticle synthesis, the silver nanoparticles completely inhibited the growth of brine shrimp at up to 100 µg/mL [135]. In plants, iron nanoparticles caused the alteration in the root cell walls structure in \textit{Arabidopsis thaliana} [136]. The structural changes were mediated by the loosening of the cells. The toxicity of the nanoparticles depends on the shape, size, and structure of the nanoparticle [137]. The dose of the nanoparticles also plays an important factor in the toxicity. Higher dose results in more toxicity as compared to the lower doses [138]. The dispersion of nanoparticles is another important factor affecting toxicity. The well-dispersed nanoparticles cause more toxicity as compared to the agglomerated nanoparticles [139]. Toxicity evaluation of the biogenic silver nanoparticles produced from \textit{Althaea officinalis} was performed. The study evaluated the toxicity imposed by the two types of silver nanoparticles prepared from the infusion of roots and extracts [140]. The study results demonstrated that the nanoparticles prepared from the extract were more toxic as compared to the nanoparticles prepared from the root infusion.

A recent study evaluated the toxicity imposed by the biogenic synthesized nanoparticles in the zebrafish and the human endothelial cells [141]. The results from the study demonstrated reactive oxygen species formation in human endothelial cells, and the induction of apoptosis was also observed. Induction of the apoptosis was supported by the increased expression of the apoptotic biomarkers. The \textit{in vivo} trials on the zebrafish resulted in the imbalanced heart rate, mortality, and cell death in the embryo. The study also confirmed the morphological changes in the tail and yolk sac of the zebrafish. The biogenic functionalized nanoparticles cause less toxicity as compared to the chemically functionalized nanoparticles. Iron oxide nanoparticle’s neurotoxicity was observed on retention in mitochondria or lysosomes [142]. In the study, three types of iron oxide nanoparticles were synthesized, and cytotoxicity in Parkinson’s disease cellular model was analyzed. The study confirmed significant toxicity on SH-SY5Y cells imposed by lysosome-targeted nanoparticles. Inhibition of AMPK caused the increased neurotoxicity in lysosome as well as mitochondria-targeted nanoparticles [143-192]. The neurotoxicity also caused the mitochondrial membrane potential alteration.

### CONCLUSION

Biogenic nanoparticles are a new concept that came into existence after the immense harmful effects of artificially synthesized nanoparticles were observed. Biogenic nanoparticles can be produced from microbes, plants, and animals. It was observed that microbes could be considered as a useful and efficient system for the biosynthesis of nanoparticles. These microbes, especially bacteria, fungi, yeast, and algae, have the potential to produce different types of nanoparti-
cles. They have been reported to produce spherical, hexagonal, cuboidal, and nanoplates. Also, the potential to produce nanoparticles utilizing various metal ions such as gold, silver, platinum, palladium, etc. When compared with the artificially designed nanoparticles, the biosynthesized nanoparticles exhibited less toxicity. The studies have reported the various mechanisms involved in the reduction of metal ions to produce nanoparticles by the microbes. The biogenic nanoparticles are produced both intracellularly as well as extracellularly. Although the biogenic nanoparticles are considered less toxic, a few recent studies have demonstrated their toxic effects on the organisms and organ systems.

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CONFLICT OF INTEREST
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REFERENCES
[1] Qin D, Dezhi Q, Guangrui Y, Yabo W, Yanbiao Z, Li Z. Green synthesis of bioactive tryptsin-conjugated Ag nanocomposite with antibacterial activity. Appl Surf Sci 2019; 469: 528-36. http://dx.doi.org/10.1016/j.apsusc.2018.11.057
[2] Wu D, GUO L, Li S-J. Synthesis, structural characterization and anti-breast cancer activity evaluation of three new Schiff base metal (II) complexes and their nanoparticles. J Mol Struct 2020; 1199126938. http://dx.doi.org/10.1016/j.molstruc.2019.126938
[3] Qi Y, Ye J, Ren S, et al. In-situ synthesis of metal nanoparticles-metal-organic frameworks: Highly effective catalytic performance and synergistic antimicrobial activity. J Hazard Mater 2020; 387121687. http://dx.doi.org/10.1016/j.jhazmat.2019.121687 PMID: 31784130
[4] Nath D, Banerjee P. Green nanotechnology - a new hope for medicine. Environ Toxicol Pharmacol 2013; 36(3): 997-1014. http://dx.doi.org/10.1016/j.etap.2013.09.002 PMID: 24095605
[5] Kharisov BI, Kharissova OV, Ortiz-Mendez U. CRC concise encyclopedia of nanotechnology. CRC Press 2016.
[6] Potara M, Boca S, Licarete E, et al. Chitosan-coated triangular silver nanoparticles as a novel class of biocompatible, highly sensitive plasmonic platforms for intracellular SERS sensing and imaging. Nanoscale 2013; 5(13): 6013-22. http://dx.doi.org/10.1039/c3nr00005b PMID: 23715524
[7] Lu Z, Xiao X, Wang L, Chen Z, et al. Self-assembly of fluorescent silver nanoclusters in hybridized DNA duplexes for single nucleotide mutation identification. J Am Chem Soc 2010; 132(3): 932-4. http://dx.doi.org/10.1021/ja907075s PMID: 20038102
[8] Chai F, Wang C, Wang T, Ma Z, Su Z. L-cysteine functionalized gold nanoparticles for the colorimetric detection of Hg2+ induced by ultraviolet light. Nanotechnology 2010; 21(2)025501 http://dx.doi.org/10.1088/0957-4484/21/2/025501 PMID: 19955605
[9] Kim J, Sadowsky MJ, Hur H-G. Simultaneous synthesis of temperature-tunable peptide and gold nanoparticle hybrid spheres. Biomacromolecules 2011; 12(7): 2518-23. http://dx.doi.org/10.1021/bm200309x PMID: 21615084
[10] Noruzi M. Biosynthesis of gold nanoparticles using plant extracts. Bioprocess Biosyst Eng 2015; 38(1): 1-14. http://dx.doi.org/10.1007/s00449-014-1251-0 PMID: 25090979
[11] Zhang L, Li Z, Guangrui Y, et al. Synthesis of Hg2+ nanocrystals in the Lysozyme aqueous solution through biomimetic method. Appl Surf Sci 2012; 258(20): 8185-91. http://dx.doi.org/10.1016/j.apsusc.2012.05.018
[12] Da-Peng Y, Shouhui C, Peng H, et al. Bacteria-template synthesized silver microspheres with hollow and porous structures as excellent SERS substrate. Green Chem 2010; 12(11): 2038-42. http://dx.doi.org/10.1039/c0gc00431f
[13] Singh P, Kim YJ, Zhang D, Yang DC. Biological Synthesis of Nanoparticles from Plants and Microorganisms. Trends Biotechnol 2016; 34(7): 588-99. http://dx.doi.org/10.1016/j.tibtech.2016.02.006 PMID: 26944794
[14] Luis C-H, Karla A-A, Jeisson U-A, et al. Green synthesis of gold and silver nanoparticles. Res J Pharm Biol Chem Sci 2015; 6(3): 1710-6.
[15] Deepak V, Umamaheshwaran PS, Guhan K, et al. Synthesis of gold and silver nanoparticles using URAK. Colloids Surf B Biointerfaces 2011; 86(2): 353-8. http://dx.doi.org/10.1016/j.colsurfb.2011.04.019 PMID: 21592748
[16] Quinteros MA, Bonilla JO, Alborné V, Villegas LB, Paez PL. Biogenic nanoparticles: Synthesis, stability and biocompatibility mediated by proteins of Pseudomonas aeruginosa. Colloids Surf B Biointerfaces 2019; 184110517. http://dx.doi.org/10.1016/j.colsurfb.2019.110517 PMID: 31605948
[17] Ottoni CA, Lima Neto MC, Léo P, Ortolan BD, Barbieri E, De Souza AO. Environmental impact of biogenic silver nanoparticles in soil and aquatic organisms. Chemosphere 2020; 239124698. http://dx.doi.org/10.1016/j.chemosphere.2019.124698 PMID: 31493753
[18] Van de Walle A, Plan Sangnier A, Abou-Hassan A, et al. Biological synthesis of magnetic nanoparticles from nano-degradation products revealed in human stem cells. Proc Natl Acad Sci USA 2019; 116(10): 4044-53. http://dx.doi.org/10.1073/pnas.1816792116 PMID: 30760598
[19] Luo C-H, Shanmugam V, Yeh C-S. Nanoparticle biosynthesis using unicellular and subcellular supports. NPG Asia Mater 2015; 7(8) e209. http://dx.doi.org/10.1038/am.2015.90
[20] Singh OV. Bio-nanoparticles: biosynthesis and sustainable biotechnological implications. John Wiley & Sons 2015. http://dx.doi.org/10.1002/9781118677629
[21] Krumov N, Perner-Nochta I, Oder S, et al. Bioremediation of inorganic nanomaterials and nanostructures. Adv Colloid Interface Sci 2013; 189-190: 1-20. http://dx.doi.org/10.1016/j.cis.2012.12.001 PMID: 23332127
Aboelfetoh EF, El-Shenody RA, Ghobara MM. Eco-friendly synthesis of silver nanoparticles using green algae (Spirulina platensis). J Nanotechnology 2015, Article ID 132675.

Sharma A, Algae as crucial organisms in advancing nanotechnology: quo vadis? Wiley Interdiscip Rev Nanomed Nanobiotechnol 2016; 8(2): 112-9.

Velgosova O, Green synthesis of Ag nanoparticles: Effect of algae life cycle on Ag nanoparticle production and long-term stability. Trans Nonferrous Met Soc China 2018; 28(5): 974-9.

Sharma B. Biosynthesis of gold nanoparticles using a freshwater green alga, Prasiola crispa. Mater Lett 2014; 116: 94-7.

Arya A, Mishra V, Chandawat TS. Green synthesis of silver nanoparticles from green algae (Botryococcus braunii) and its catalytic behavior for the synthesis of benzimidazoles. Chemical Data Collections 2019; 20100190.

Fatima R, Priya M, Indurthi L, Radhakrishnan V, Sudhakaran R. Biosynthesis of silver nanoparticles using red algae Portieria hornemannii and its antibacterial activity against fish pathogens. Microb Pathog 2020; 138103780.

Namvar F, Green synthesis and characterization of gold nanoparticles. Bioimpacts 2011; 1(3): 149-52.

Lee JY, Wang DI, Ting YP. Identification of active biomolecules in the high-yield synthesis of single-crystalline gold nanoparticles in algol solutions. Small 2007; 3(4): 672-82.

Clements S, Roberta B, Jérémie M, et al. Nano-gold biosynthesis by silica-encapsulated micro-algae: A “living” bio-hybrid material. J Mater Chem 2010; 20(42): 9347-9.

Shakibaie M, Forootanfar H, Mollazadeh-Moghaddam K, et al. Bioconversion and catalytic and antibacterial activities. Environ Monit Assess 2017; 189(7): 349.

Sharma B. Biosynthesis of silver nanoparticles using a freshwater green alga, Prasiola crispa. Mater Lett 2014; 116: 94-7.
Microorganisms as Nano-factories for the Synthesis of Metal Nanoparticles

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n nanoparticles synthesized by Bacillus steareothermophilus. Biosen Bioelec, 2014; 54: 217-221. doi:10.1016/j.bios.2013.11.013

[165] Mabbett AN, Yong P, Farr JP. Macaskie LE. Reduction of Cr(VI) by "palladized" biomass of Desulfovibrio desulfuricans ATCC 29577. Biotech Bioeng 2004; 87(1): 104-109. doi:10.1002/bit.20055

[166] Malarkodi C, Rajeshkumar S, Vanaj M, Paulkumar K, Gnanajobitha G, Annadurai G. Eco-friendly synthesis and characterization of gold nanoparticles using Klebsiella pneumoniae. J Nanostruct Chem 2013; 3(1): 2013.

[167] Manjunath HM, Joshi G, Raju NG. Biofabrication of gold nanoparticles using marine endophytic fungus - Penicillium citrinum. IET Nanobiotechnol 2017; 11(1): 40-44. doi:10.1049/iet-nbt.2016.0065

[168] Markus, J., Mathiyalagan, R., Kim, et al. Intracellular synthesis of gold nanoparticles with antioxidant activity by probiotic Lactobacillus kimchicus DCY51T isolated from Korean kimchi. Enzyme Microb Technol, 2016; 95: 85-93. doi:10.1016/j.enmictec.2016.08.018

[169] Mishra, A., Tripathy, S. K., Wahab, R., Jeong, S. H., Yun, S. I. Fungus mediated synthesis of gold nanoparticles and their conjugation with genomic DNA isolated from Escherichia coli and Staphylococcus aureus. Process Biochemistry 2012; 47(5): 701-711. doi:10.1016/j.procbio.2012.01.017

[170] Mohammed Fayaz A, Girilal M, Rahman M, Venkatesan R, Mishra, A., Tripathy, S. K., Wahab, R., Jeong, S. H., Hwang, I., Doktycz, M.J. Biofabrication of discrete spherical gold nanoparticles by the fungus Penicillium chrysogenum. Int J Nanosci Nanotechnol 2011; 7: 102-105.

[171] Narayanan KB, Sukthivel N. Myocystallization of gold ions by the fungus Cylindrocladium floridanum. World J Microbiol Biotechn 2003; 29(10): 2207-2211. doi:10.1007/s00253-011-3556-0

[172] Oves M, Khan MS, Zaidi A, Ahmed AS, Ahmed F, Azam A. Antibacterial and cytotoxic efficacy of extracellular silver nanoparticles biofabricated from chromium reducing novel OS4 strain of Stenotrophomonas maltophilia. PLOS ONE 2013; 8(3): e59140. doi:10.1371/journal.pone.0059140

[173] Parah HJ, Jung C, Lee JH, Park H.G. A gold nanorod-based optical DNA biosensor for the diagnosis of pathogens. Biosensors and Bioelectronics 2010; 26(2); 667-673. doi:10.1016/j.bios.2010.06.067

[174] Park K, Hsiao MS, Yi YJ, Izor S, Koerner H, Jawaid A, Vaia RA. Highly Concentrated Seed-Mediated Synthesis of Monodispersed Gold Nanorods. ACS App Mat Inter 2017; 9(31), 26365-71. doi:10.1021/acsami.7b08003

[175] Philip, D. Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract. Spectrochimica Acta - Part A: Mol Biomol Spect 2009; 73(2): 378-381. doi:10.1016/j.saa.2009.02.037

[176] Priyadarshini E, Pradhan N. Gold nanoparticles as efficient sensors in colorimetric detection of toxic metal ions: A review. Sensors Actuators, B: Chemical 2017; 238: 888-902. doi:10.1016/j.snb.2016.06.081

[177] Quester K, Borja A, Nestor ARV, Lopez MAC, Longoria E.C. SERS properties of different sized and shaped gold nanoparticles biosynthesized under different environmental conditions by Neurospora crassa extract. PLOS ONE. 2013; 8: e77486.

[178] Rajathi FAA, Parthiban C, Kumar VG, Anantharaman P. Green synthesis of silver nanoparticles from deoiled brown algal extract via Box-Behnken based design and their antimicrobial and sensing properties De Gruyter 2012; 99: 166-173.

[179] Sarkar J, Ray S, Chattopadhyay D, Laskar A, Acharya K. Mycoparticles of fungus nanoparticles using a phytopathogen Alternaria alternata. Biopros Biosys Eng 2012; 35(4): 637-643. doi:10.1007/s11004-011-9646-4

[180] Satyanarayana RA, Chen CY, Chen CC, Jean JS, Chen HR, Wang J.C. Biological synthesis of gold and silver nanoparticles mediated by the bacteria Bacillus subtilis. J Nano Nanotech 2010; 10(10): 6567-6574. doi:10.1016/j.nanoj.2010.06.051

[181] Sayed ET, Barakat NAM, Abdelkareem MA, Fouad H, Nakagawa N. Yeast extract as an effective and safe mediator for the baker's-yeast-based microbial fuel cell. Industrial and Engineering Chemistry Research, 2015; 54(12): 3116-3122. doi:10.1021/ie5042352

[182] Shahverdi AR, Minaeein S, Shahverdi HR, Jamalifa H, Nohi AA. Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: A novel biological approach. Process Biochem, 2007; 42(5): 919-923. doi:10.1016/j.procbio.2007.02.005

[183] Sheikhloo Z, Salouti M. Intracellular biosynthesis of gold nanoparticles by the fungus Penicillium chrysogenum. Int J Nanosci Nanotechnol 2011; 7: 102-105.

[184] Sheikhloo Z, Salouti M, Katiraece F. Biological Synthesis of Gold Nanoparticles by Fungus Epicoccum nigrum. J Cluster Sci 2011; 22(4): 661-665. doi:10.1007/s10876-011-0412-4

[185] Srivastava SK, Yamada R, Oñino C, Kondo A. Biogenic synthesis and characterization of gold nanoparticles by Escherichia coli K12 and its heterogeneous catalysis in degradation of 4-nitrophenol. Nanoscale Res Lett 2013; 8.

[186] Suresh AK, Pelletier DA, Wang W, Broich ML, Moon JW, Gu B, Doktycz, M.J. Biofabrication of discrete spherical gold nanoparticles using the metal-reducing bacterium Shewanella oneidensis. Acta Biomaterialia 2011; 7(5): 2148-2152. doi:10.1016/j.actbio.2011.01.023

[187] Vala, A. K. Exploration on green synthesis of gold nanoparticles by a marine-derived fungus Aspergillus sydowii. Envir Prog Sustainable Energy, 2015; 34(1): 194-197.

[188] Verma VC, Singh SK, Solanki R, Prakash, S. Biofabrication of anisotropic gold nanotriangles using extract of endophytic and characterized by environmental isolate of Acinetobacter sp. SW30. World J Microbiol Biotechnol 2014; 30(10): 2723-31. doi:10.1007/s11274-014-1696-y

[189] Yamal, G., Sharmila, P., Rao, K. S., & Pardha-Saradhi, P. (2013). Yeast Extract Mannitol medium and its constituents promote synthesis of Au nanoparticles. Process Biochem 2013; 48(3): 532-8. doi:10.1016/j.procbio.2013.02.011