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Biological Synthesis of Nanoparticles Using Endophytic Microorganisms: Current Development

Omar Messaoudi and Mourad Bendahou

Abstract

Nanotechnology is a new emerging interdisciplinary approach created by pairing of engineering, chemical, and biological approaches. This technology produces nanoparticles using different methods of traditional physical and chemical processes; however, the outlook in this field of research is to use ecofriendly, nontoxic, and clean methods for the synthesis of nanoparticles. Biological entities, such as plants, bacteria, fungi, algae, yeast, and actinomycetes, are the best candidate to achieve this goal. Among the biological route, those involve endophytic microorganisms to reduce metallic ions into nanoparticles. This method is considered as an attractive option and can open a new horizon on the interface of biology and nanotechnology. The present chapter highlights the latest research about endophytic microorganisms and their application in the synthesis of nanoparticles, as well as the mechanisms involved in the formation of nanoparticles.

Keywords: endophyte microorganisms, green nanotechnology, nanoparticles

1. Introduction

Nanotechnology is a new emerging interdisciplinary approach of created by pairing of biotechnology, and nanotechnology [1]. This new technology produced nanoparticles of various types (silver, copper, zinc, gold, etc.) at the nanoscale level (less than 100 nm). Three different methods can be employed for the synthesis of nanoparticles, including, chemical, physical and biological methods. These three methods follow either the bottom-up approach, or the top-down approach for the synthesis of nanoparticles [2].

The outlook in this field of research is to use ecofriendly, nontoxic and clean method for the synthesis of nanoparticles [3]. The chemical and physical methods are generally expensive and associated with destructive effects on the environment and human health [4]. In order to counter those limitations, one of the proposed solution is the application of a novel route for producing nanoparticles based on bottom-up method, called 'green synthesis', which is regarded as an important tool and gaining great attention in current research. This method is based on the utilization of natural resource, such as plants, fungi, bacteria, actinomycetes, yeast and algae, to reduce the metal ions into metallic nanoparticles [5].

The green synthesis of nanoparticles offers a set number of benefits compared with physical and chemical methods, since this method is cost-effectively, eco-friendly, uses less energy and can provide nanoparticles with better defined size and morphology, with a great compatibility for pharmaceuticals, medical, agronomical and environmental applications [6].

Microbial-mediated biosynthesis of nanomaterials is one of the promising biological-based nanomanufacturing process [7]. Microorganisms can produce nanoparticles by intracellular or extracellular synthesis, according to the location where nanoparticles are formed, through enzymes or biomolecules generated by the cell activities [8]. The use of microorganisms offers different advantages over the biosynthesis of nanoparticles by plants and algae, since microorganism can be easily scale-up, and they offer the possibility to changing culture condition to obtained nanoparticles with desired shape and sizes [9].

One approach that shows immense potential is based on the biosynthesis of nanoparticles using endophytic microorganisms, which is considered as a new potential source, under explored [10]. In this chapter, we present, the latest research about nanoparticles from endophytic microorganisms.

2. Endophytic microorganism: bacteria and fungi

“Endophytes” is a Greek word that mean “within plant”, this term is used for microorganisms (bacteria or fungi) that dwell within plant tissues, without causing any disease, infection, or damage to the plant tissues [11]. Every plant host, intercellularly and/or extracellularly, in various spaces of plant parts including roots, leaves, stems, flowers, and seeds, one to more endophytes microorganisms [12]. To date, endophytes microorganism has been found in all plant species that exist on the earth (nearly 390,000 plants) [13]. Mutualist is the most common relationship between plants and endophytes, however, in some cases and under some conditions, the endophytes can behave as opportunistic pathogens [14].

To have a stable symbiotic relationship, the plant host provides to endophytes the necessary organic nutrient, generated through photosynthesis, for growth and multiplication [14]. On the other side the endophytes offer different beneficial effects to the host plant, this including: (i) nutrient assimilation: by synthesis of iron (Fe)-sequestering siderophores, fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus [15]. (ii) Stimulation of plant growth: by secretion of plant growth regulators (PGRs), such as auxin, cytokinin, ethylene and gibberellin [16]. (iii) Protection of host plants from attack of pathogens microorganisms and insects: through secretion of various bioactive secondary metabolites as well as lytic enzymes [17].

The endophytes microorganisms can be acquired directly from the environment (horizontal transmission), or are vertically transmitted from generation to generation via seed [18]. The majority of endophytes are acquired via the first mechanism of transmission, this was confirmed through the study of the diversity of microorganisms in seeds and seedlings, raised under sterile conditions, which are typically lower than the diversity of microorganisms in plants grown in soil [19].

Endophytes are studied under two categories, bacterial endophytes and fungal endophytes [20]. The structure of the microorganism communities resides inside the plants, depends on several factors, including, the nature of soil and the plant host species [21]. To study the composition in microorganisms of endophytes, the culture-dependent methods do not allow a complete overview of the endophytic population, because the uncultured microorganisms cannot be recovered and

identified using this method. However, the use of molecular approaches, including high throughput techniques of next generation sequencing (NGS), confers a rapid analysis of the composition and diversity of plant microbial endophytes communities [22]. According to the study of Hardoim et al., 2014 [14], which analyze the sequences of 16 s DNAr assigned to endophytic bacteria strains, including cultured and uncultured bacteria, he found that, 96% of analyzed sequences belong to four different cultured phyla, which is reported to be dominant in the plant environment, including: 54% *Proteobacteria*, 20% *Actinobacteria*, 16% *Firmicutes*, and 6% *Bacteroidetes*. However, 19 phyla belong to the non cultured bacteria. Furthermore, 50% of the analyzed sequences, which are the predominant endophytes strains, belong to the genera, *Pseudomonas*, *Enterobacter*, *Pantoea*, *Stenotrophomonas*, *Acinetobacter*, and *Serratia*, all these genera are member within the class of *Gammaproteobacteria* (*Proteobacteria* phylum). Other genera are also well represented within endophytic bacteria population, this including *Streptomyces*, *Microbacterium*, *Mycobacterium*, *Arthrobacter*, as well as *Bacillus*, *Paenibacillus*, and *Staphylococcus*.

Endophytic fungi are ubiquitous in plants and are mainly members of *Ascomycota* or their mitosporic fungi, as well as some taxa of *Basidiomycota*, *Zygomycota*, and *Mucoromycota* [23]. Li et al. [24], examined endophytic fungi associated with the stem and root of 10 halophytic species colonizing the Gurbantonggut desert, they obtained 36 endophytic fungal taxa, dominated by *Alternaria eichhorniae*, *Monosporascus ibericus*, and *Pezizomycotina* sp. 1. However, a total of 56 endophytic fungi was isolated from leaf and root segments of *Salvia abrotanoides* at the three sites by Teimoori-Boghsani et al. [25]. The isolated strains belong to 16 different fungal genera, this including: *Penicillium*, *Paraphoma*, *Phaeoacremonium*, *Talaromyces*, *Aspergillus*, *Psathyrella*, *Trichoderma*, *Alternaria*, *Thielavia*, *Acremonium*, *Fusarium*, *Talaromyces*, *Coniolaria*, *Paecilomyces*, *Simplicillium*, and *Monocillium*. Among the obtained strains, only two isolates were recovered from the plant's leaves (*Thielavia microspore* and *Aspergillus* sp.), while the remaining isolates were obtained from root samples.

3. The green nanotechnology

Nanotechnology is a rapidly growing field of science, and can be defined as the manipulation of materials at the nanometer scale or one billionth of a meter. It's become an integral part of the biotechnology and regarded as one of the key technologies [26].

Nanotechnology produces materials which have one dimension less than 100 nm at least, these materials, called nanoparticles, can be produced using different metals, such as: gold (Au), silver (Ag), copper oxide (CuO), zinc oxide (ZnO), iron (Fe₂O₃), palladium (Pd), platinum (Pt), nickel oxide (NiO), magnesium oxide (MgO), selenium (Se) and titanium dioxide (TiO₂) [27].

The synthesis of nanoparticles is based on two approaches: (1) top-down approach and (2) bottom-up approach (**Figure 1**) [28]. The first approach (top-down approach) is destructive method, based on the decomposition of larger molecule into smaller units, these unit are then converted into appropriate nanoparticles. Several physical methods are applied in this case: mechanical milling, chemical etching, sputtering, laser ablation electro-explosion [29]. The second approach (bottom-up approach), is employed in reverse to the first approach, in fact, in this case, nanoparticles are formed when atoms are self assemble together [30]. The synthesis of nanoparticles using this approach, can be carried

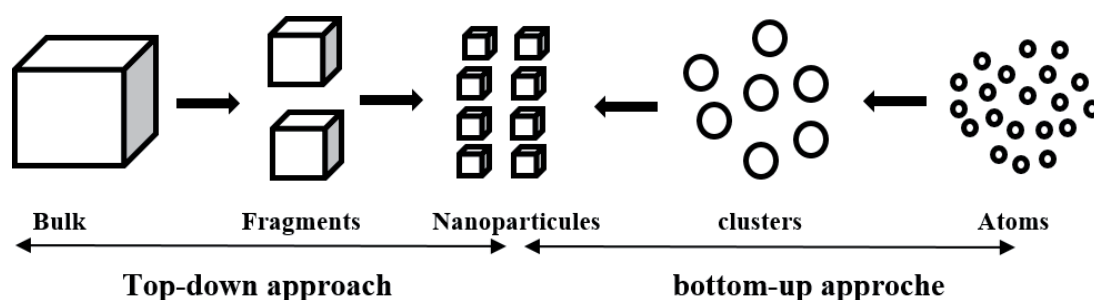


Figure 1.
Top-down and bottom-up approach.

out by several physical and chemical methods including: spinning, template support synthesis, plasma or flame spraying synthesis, laser pyrolysis, CVD, atomic or molecular condensation [31]. Biological routes can also be applied to reduce metallic ions into neutral atoms (zero valent atoms) for synthesis of nanoparticles with bottom-up approach, this method is so called green nanotechnology, in this case several biological sources, available in nature, are involved, such as: (i) utilization of microorganism (bacteria, fungi); (ii) utilization of plant extracts; (iii) utilization of microseaweeds; (iv) using enzymes and biomolecules [32, 33].

Biological agents involved in green nanotechnology offer many benefits as compared with physical and chemical syntheses, in fact, these techniques are costly, requires higher utilization of energy, and utilize toxic chemicals that may have a disastrous effect on the environment [34]. In contrast, biological approach has several edges over chemical and physical methods for synthesis of nanoparticles, as it is low cost, eco-friendly, non-toxic, clean and can be scaled up to larger-scale synthesis with ease [35].

Biological nanoparticles, synthesized using different metal, have been applied in many fields, in fact, the silver nanoparticles are widely used in medical fields, for example Al-Sheddi et al. [36], show the potential of silver nanoparticles synthesized using an extract of *Nepeta deflersiana* against Human Cervical Cancer Cells (HeLA). However, Soliman et al. [37] indicate that the silver nanoparticles synthesized by the pink yeast, *Rhodotorula* sp. ATL72, isolated from salt marches near mediterranean sea, Egypt, exhibited strong antimicrobial activity against a wide range of Gram positive and Gram negative bacteria as well as fungi with low MIC value. Moreover, zinc and titanium nanoparticles are generally used in cosmetics fields [38]. Biological nanoparticles can also apply as sensors for various biomolecules related to environmental factors and agriculture, as well as they can also use for gene delivery and cell labeling in plants and in medicine [39].

4. Mechanisms of nanoparticle biosynthesis by microorganisms

Although, the number of studies which elucidate the green synthesis of nanoparticles using microorganisms, there is a little work about the mechanism and the biochemical pathway involved behind the synthesis of metal nanoparticles.

Intra and extra cellular microbial enzymes and secondary metabolites secreted by microorganisms, play a key role in the reduction of metal ions into their respective nanoparticles. In fact, It has been found that the microorganisms when are exposed to metal ion solution, they are responding to this environmental stress by the secretion of enzymes and biomolecules that possess a reducing potential of metal salts, consequently the metal ions are detoxified to less toxic metal nanoparticles [5].

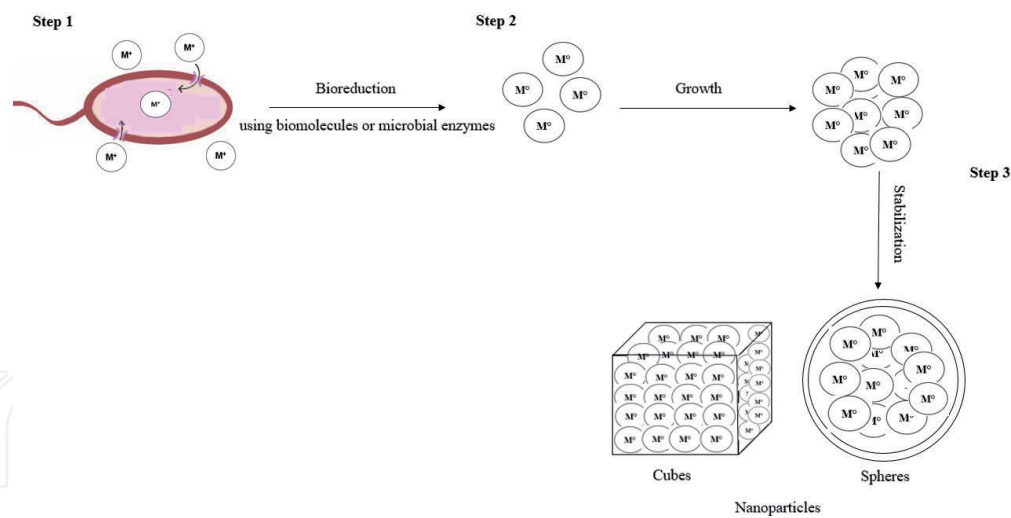


Figure 2.
Mechanisms of nanoparticle synthesis.

Three steps are involved in the biosynthesis of nanoparticles by microorganisms (**Figure 2**):

- In the first step, metallic ions are captured on the surface of microbial cells via electrostatic interaction with the negatively charged cell wall, or they are absorbed inside the microbial cells, through cationic membrane transport systems that normally transport metabolically important cations [5, 40].
- In the second step, metallic ions (M^+) are bioreduced into zero-valent metals (M^0). This reaction can be catalyzed by: (i) the active groups, such as the hydroxyl group (C-OH) or the ionized carboxyl (COO⁻) group, of biomolecules biosynthesized by the microorganisms having reduction capabilities., or (ii) or by microbial enzymes, such as, NADH-dependent nitrate reductase, which catalyze the reduction of silver ions to silver nanoparticles at pH 7.2, using NADH as electron source and 8-hydroxyquinoline as electron shuttle [41, 42]. As results of this reduction, the metal ions are changed from their mono- or divalent oxidation states to reduced metal ions (zero-valent states). Afterward, the nanoparticles joint to form different morphology shapes such as, spheres, hexagons, triangles, cubes, ovale, etc. [43].
- The third step corresponding to the stabilization of nanoparticles with capping agents, to prevent further growth and agglomeration and controlling the shape and size of the biosynthesized nanoparticles [5].

The size of nanoparticles biosynthesize by endophytic microorganisms affect the activity, it has been proved that nanoparticles with small size provide great surface/volume ration and guarantee a good activity [44]. Different physico-chemical parameters should be controlled and optimized, such as, temperature, pH, metal salt concentration, incubation period, agitation, nature and concentration of carbon and nitrogen source in culture media, to producing homogeneous nanoparticles in size and shape, with satisfied activity [38].

5. Nanoparticles synthetized by endophytic microorganisms

Biological methods are being a popular trend in the synthesis of metal nanoparticles. Among them, those involving saprophytic microorganisms (bacteria and

fungi), which are able to turn the metal ions, from their environment, into metallic nanoparticles through enzymes and secondary metabolites generated by the cell activities. This process provides greater stability and appropriate dimensions of synthesized nanoparticles [37].

Compared with saprophytic microorganisms, the application of endophytic microorganisms has emerged as a novel research area for the green synthesis of nanoparticles. This field of research can open a new horizon, on the interface of biology and nanotechnology, for novel nanomaterials with diverse applications [45].

Different endophytic microorganisms, including fungi, bacteria and actinomycetes, can be used for the biosynthesis of nanoparticles from different metal, such as silver, gold, zinc, copper, etc. **Table 1** summarizes the recent researches in this field.

5.1 Nanoparticles synthesized by endophytic bacteria

Some endophytic bacteria, have developed a specific defense mechanism to overcome toxicity of metal ions, this mechanism is based on the precipitation of ions metals at the nanometer scale to produce nanoparticles [63]. It was observed that some of endophytic bacteria could survive and grow even at high metal ion concentrations. Bacteria possess such remarkable ability to reduce metal ions into nanoparticles, can be a good candidate for nanoparticles synthesis [64].

Ibrahim et al. [46, 47] reported the isolation of *Bacillus siamensis* C1 from *Coriandrum sativum* and *Pseudomonas poae* CO from *Allium sativum*, both strains produce silver nanoparticles with spherical shape and exhibited potential antibacterial, antibiofilm and antifungal activity.

Gold nanoparticles with spherical form and size range from 5 to 50 nm, has been successfully synthesized by the endophytic bacteria, *Pseudomonas fluorescens* 417, isolated from the plant, *Coffea arabica*. The synthesized gold nanoparticles show bactericidal activity against a panel of clinically significant pathogens [49]. The same author, Syed et al. [48], use the strain *Aneurinibacillus migulanus*, isolated from surface sterilized inner leaf segment of *Mimosa pudica*, for the biosynthesis of silver nanoparticles with different shapes, including, spherical, oval, cubic and triangular shapes. The particle size has been determined by Dynamic Light Scattering (DLS) method, and revealed average size of 24.27 nm. The bactericidal activity of the biosynthesis silver nanoparticles indicates interesting activity against both Gram-positive and Gram-negative pathogenic bacteria. The highest activity was observed against *Pseudomonas aeruginosa*, which is considered as clinically important bacteria.

5.2 Nanoparticles synthesized by endophytic fungi

In recent years, the utilization of endophytic fungi for the production of metallic nanoparticles has attracted more attention, due to their metal tolerance, metal uptake and accumulation capability [65]. Compared with the other microorganisms, fungi are good machines for the synthesis of any type of metallic nanoparticles, and can provide a several advantages, such as: (i) Easy for isolation from soil or plants, compared with rare bacteria and actinomycetes, which required specific enrichment methods for isolation [56]. (ii) Secrete large amounts of metabolites and extracellular enzymes, which facilitate the reduction of metal ions into nanoparticles. (iii) Easy to scale-up, since they have a rapid growth [66] (iv). Most of the fungi have a large range of growth for pH, temperature and NaCl, which facilitate the change of culture conditions in order to produce homogeneous nanoparticles [67].

Plants	Endophytes	Shapes	Size	Types of NPs	Activity	References
<i>Coriandrum sativum</i>	<i>Bacillus siamensis</i> C1	Spherical	25–50 nm	Silver	Antibacterial	[46]
<i>Allium sativum</i>	<i>Pseudomonas poae</i> CO	Spherical	19.8–44.9 nm	Silver	Antifungal	[47]
<i>Mimosa pudica</i>	<i>Aneurinibacillus migulanus</i>	Spherical, oval, cubic, triangular	~ 24.27 nm	Silver	Antibacterial	[48]
<i>Coffea arabica</i>	<i>Pseudomonas fluorescens</i> 417	Spherical	5–50 nm	Gold	Antibacterial	[49]
<i>Raphanus sativus</i>	<i>Alternaria</i> sp.	Spherical	4–30 nm.	Silver	Antibacterial	[35]
<i>Taxus baccata</i>	<i>Nemania</i> sp.	Spherical or ellipsoidal	5–70 nm	Silver	Antibacterial	[50]
<i>Erythrophleum fordii</i>	<i>Alternaria tenuissima</i>	Spherical	15–45 nm.	Zinc oxide	Antimicrobial, anticancer and antioxidant	[51]
<i>Chonemorpha fragrans.</i>	<i>Fusarium solani</i>	Spindle	40–45 nm	Gold	Anticancer	[52]
<i>Cinnamomum zeylanicum</i>	<i>Lasiodiplodia theobromae</i>	Spherical to oval	~ 76 nm	Silver	Antibacterial	[53]
<i>Chiliadenus montanus</i>	<i>Trichoderma atroviride</i>	Spherical	10 to 15 nm.	Silver	Antibacterial	[54]
<i>Madhuca longifolia</i>	<i>Pestalotia</i> sp.	Angular	< 40 nm	Silver	Antibacterial	[55]
<i>Pinus densiflora</i>	<i>Talaromyces purpureogenus</i>	Round to triangle	~ 25 nm	Silver	Antimicrobial and anticancer	[56]
<i>Ocimum tenuiflorum</i>	<i>Exserohilum rostrata,</i>	Spherical	10–15 nm	Silver	Antibacterial, antiinflammatory, and antioxidant	[57]
<i>Borszczowia aralocaspica</i>	<i>Isoptericola</i> SYSU 333150	Spherical	11–40 nm	Silver	Antibacterial	[58]
<i>Oxalis corniculata</i>	<i>Streptomyces zaomyceticus</i> Oc-5	Spherical	~78 nm	Copper	Antimicrobial, antioxidant and anticancer	[59]
<i>Mentha longifolia</i>	<i>Streptomyces</i> sp.	Spherical	2.3–85 nm	Silver	Antimicrobial	[60]

Plants	Endophytes	Shapes	Size	Types of NPs	Activity	References
<i>Convolvulus arvensis</i>	<i>Streptomyces capillispiralis</i> Ca-1,	Spherical	3.6–59 nm	Copper	Antimicrobial and insecticides	[61]
<i>Ocimum sanctum</i>	<i>Streptomyces coelicolor</i>	Spherical and ellipsoidal	~25 nm	Magnesium	Antimicrobial	[62]

Table 1.
Biosynthesis of nanoparticles from endophytic microorganisms with their respective size and biological activity.

Clarance et al. [52], reported the isolation of the endophytic fungi, *Fusarium solani*, from the plant *Chonemorpha fragrans*, which is used for the biosynthesis of gold nanoparticles. The morphology of synthesized nanomaterials was found to have needled and flower like structures with spindle shape, and showed pink-ruby red colors and high peak plasmon band between 510 and 560 nm. The gold synthesized nanoparticles showed cytotoxic activity against cervical cancer cells (HeLa) (IC₅₀: 0.8 ± 0.5 µg/mL) and human breast cancer cells (MCF-7) (IC₅₀: 1.3 ± 0.5 µg/mL).

Abdelhakim et al. [51], use the culture filtrate of the endophytic fungi *Alternaria tenuissima*, isolated from *Erythrophleum fordii*, to produce zinc oxide nanoparticles. The shape of the biosynthesized nanoparticles was spherical and having size diameter ranges between 15 and 45 nm along with significant antimicrobial, anticancer and antioxidant activity.

The endophyte *Exserohilum rostrata* has been isolated from the plant *Ocimum tenuiflorum* by Bagur et al. [57], this strain was used for the biosynthesis of spherical silver nanoparticles with a size, range between 10 and 15 nm, and showed significant antimicrobial activity and other biological properties such as, anti-inflammatory, and antioxidant activities.

5.3 Nanoparticles synthesized by endophytic actinomycetes

Actinomycetes are Gram positive bacteria with high G + C, belong to the phylum of *Actinobacteria*, which is one of the largest taxonomic rank within the domain of *Bacteria* [68, 69]. This group of microorganisms is known by the production of a wide range of bioactive secondary metabolites. In fact, 70–80% of secondary metabolites in current clinical use, including, antibiotics, antifungals, immunosuppressives, anticancer, insecticides and antivirals, have been isolated and characterized from several species of actinomycetes, particularly from the genus *Streptomyces* [70].

Nanoparticles from endophytic actinobacteria is an emerging field yet to be established, in fact, when compared with fungi and the other bacteria, only few publications have been reported. Most of the articles about nanoparticles from endophytic actinomycetes, reporting the synthesis of nanoparticles using endophytes belong to the genus of *Streptomyces*, however, nanoparticles synthesized by rare actinobacteria have been reported in a few papers [60, 62].

The author, Hassan et al. [59, 61], publishes two papers about the utilization of endophytic *Streptomyces* for the biosynthesis of nanoparticles. In fact, they report the isolation of *Streptomyces zaomyceticus* Oc-5 and *Streptomyces capillispiralis* Ca-1, from the plants *Oxalis corniculata* and *Convolvulus arvensis* respectively. Both strains were used for the synthesis of copper nanoparticles, which exhibited different biological activity, including, antimicrobial, antioxidant and anticancer, and insecticides.

In another study, Dong et al. [58], use a rare actinobacteria, in order to control the disease caused by *Staphylococcus warneri* which have a significant impact on human health. The researchers use the strain, *Isoptericola SYSU 333150*, isolated from the plant *Borszczowia aralocaspica*, for the biosynthesis of silver nanoparticle using photo-irradiation with sunlight exposition for different periods, they obtained spherical nanoparticles with a size range between, 11–40 nm, which exhibit antimicrobial activity against the pathogen *S. warneri*.

Several others studies confirm that nanoparticles from different metallic natures, sizes and shapes, synthesized by endophytic microorganisms, are attractive options, since they exhibited various pool of biological activities, including, antimicrobial, cytotoxic, antiinflammatory, antioxidant [35, 50, 53–56, 60, 62].

6. Methods for the isolation of endophytic microorganism and the characterization of synthesized nanoparticles

The isolation methods of endophyte aim to obtain microorganisms residing within plant hosts without causing disease symptoms. The isolation protocol followed depends on several factors such as, the target group of endophyte microorganisms you would like to isolate (bacteria, fungi and Actinobacteria), species of the host plant, the part of plant tissue, sampling season, culture conditions, etc. [71].

The first step consists on surface sterilization of host plant to remove all the surface-living microorganisms [72]. Several methods can be applied, among them, the plant parts will be immersed sequentially, in several solutions of sterilization, including, 70% ethanol for 5 minutes, followed by (3–10%) of sodium hypochlorite for 2 minutes, and then immersed in hydrogen peroxide (H₂O₂) for 1 minute [73]. The final step of sterilization consists to rinse the different plant parts with distilled water three times, and soaked in 10% NaHCO₃ to inhibit fungal growth [74].

After surface sterilization, the sterilized tissue samples are cut into small pieces of 1 cm³, under sterile conditions, and then placed on tryptic soy agar plates followed by incubation for 14 days to verify the sterilization effectiveness. Afterwards, the plant segments are grinding in sterile conditions, and then the samples are serially diluted up to 10⁻³ with sterile water [75]. Aliquots of 100–200 µL of the dilutions will be spread-plated onto a series of appropriate isolation media (depend on the type of endophytic microorganisms). The appeared colonies are transferred to a new culture medium to obtain a pure culture [76]. The endophytic strains are subjected to molecular identification based on sequencing of 16 s rDNA for bacteria, and 18 s rDNA for fungi.

For nanoparticles synthesis, the endophytic strains are culturing in rotating shaker under optimum culture conditions, including: appropriate culture medium, pH, temperature, agitation. After incubation, the culture is centrifuged to separate the biomass from the supernatant [48]. Both supernatant and biomass are tested for nanoparticles synthesis, in fact microorganisms are able to synthesize nanoparticles extracellularly or intracellularly (**Figure 3**) [77].

For extracellular synthesis of nanoparticles, the obtained supernatant is mixed with a filter-sterilized metal salt solution (e.g. AgNO₃), the mixture is incubated again, the color changing, of the mixture after incubation, can indicate the synthesis of nanoparticles [78]. For example, for silver nanoparticles, the color changes from colorless to deep brown, whereas, for gold nanoparticles, it changes from ruby red to a deep purple color. Afterward, the precipitate of nanoparticles formed can be recovered by centrifugation, washed several times with distilled water and collected in the form of a bottom pellet [79].

For intracellular synthesis of nanoparticles, the biomass obtained after centrifugation, is washed several times with distilled water to remove the traces of culture medium, then mixed with a filter-sterilized solution of metal salt [80]. The synthesis of nanoparticles can be monitored by color change after the incubation period [81]. The nanoparticles synthesized inside the cell can be released after break down the cell wall by repeated cycles of ultrasonication. The nanoparticles can be purified from cellular debris, after repeated cycles of centrifugation/washing with distilled water [82].

Physicochemical characterization of nanoparticles is performed to determine the morphology, surface area, porosity, particle size and distribution, aggregation, crystal structure (crystallinity), zeta potential, structural properties and others parameters of biosynthesized nanoparticles [40].

In order to analyse the physicochemical properties of nanoparticles, different characterization techniques are applied. This includes the following:

- The formation of nanoparticles can be confirmed by spectra analysis of absorption in the wavelength range between 200 and 800 nm [83].
- The morphology, size and distribution of nanoparticles can be determined by Transmission Electron Microscopy (TEM), as well as Scanning Electron Microscopy (SEM), since morphological features significantly affect the activity of nanoparticles [84].
- The X-ray diffraction (XRD), can be used for the determination of the structural properties of nanoparticles, such as the chemical composition and the crystallinity of synthesized nanoparticles [85].
- FTIR (Fourier transform infrared) spectroscopy, is performed to identify the functional groups present on nanoparticles [86].

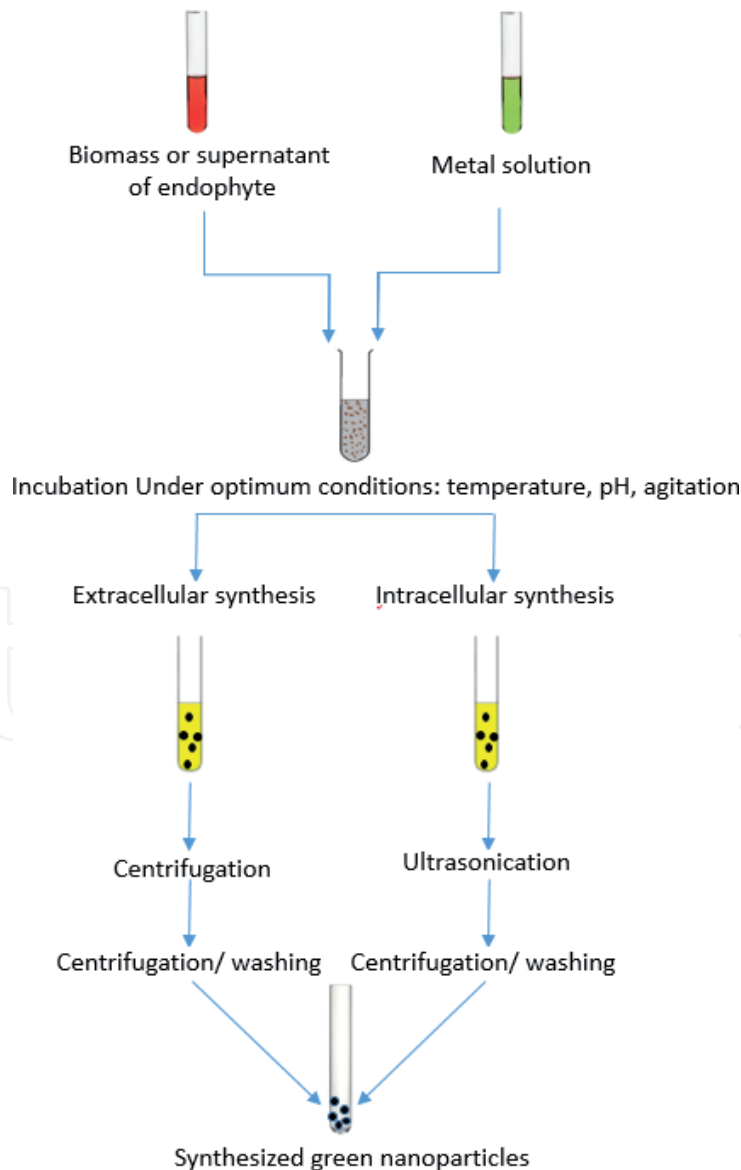


Figure 3.
Green synthesis of nanoparticles using endophyte microorganisms.

- Particle size can be estimated using, dynamic light scattering (DLS), which can be used to find the nanoparticles size at extremely low level [85].
- Surface area characterization, including, stability and surface charge of colloidal nanoparticles are evaluated by zeta potential analysis using a Zetasizer nanomachine [87].

7. Conclusion

Soil microorganisms have been largely explored as a source for nanoparticle biosynthesis; however, few reports are available about the utilization of endophytic microorganisms for synthesizing nanoparticles, and therefore, it is important to focus research in this promising biological route of nanoscience. However, since most of the endophytic microorganisms are uncultivated, it's important to concentrate researches in the development of innovating methods for the isolation of this group of microorganisms for further advancement of green synthesis of metal nanomaterials. Additionally, the mechanisms involved in the reduction and stabilization of nanoparticles, using microorganisms, is not well defined, and more elaborated studies are needed to determine all the enzymes and biomolecules involved in the nanoparticle biosynthesis.

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