Green Nanoparticles: Biogenerators; Mechanistic Aspects of Biosynthesis; Potential Applications and Future Prospective

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Nano-science is a new scientific program that demands materials and apparatus capable of manipulating chemical and physical properties of a substance at molecular scale. Now there is a processing need to develop credible, probable, and eco-friendly schedule for manufacturing a broad framework of metal and metal oxide nanoparticles. The biogenic synthesis of nanoparticles via nano-bio-technology based techniques has the effort to recognize immaculate manufacturing technologies. These new pure technologies can safely minimize circumferential pollution and dwindling the riskiness to humanitarian health brings from the use of poisonous chemicals and solvents actually used in traditional manufacturing processes. Now, green prepared nanoparticles are of considerable concern in the area of biology and medicine due to their shape and unparalleled particle size according to and their biological, physical and chemical properties. Most of the previous studies use biomolecules (carbohydrates, amino acids, proteins and sugars), various types of whole cells of different microorganisms (algae, fungi and bacteria), or mixed plant resources for the formation of metal nanoparticles. Moreover, the biological program, particularly, the use of natural organisms provides a reliable, simple, non-toxic and environmentally friendly method. Hence, the present review provides a brief overview of the biogenic synthesis, potential applications of the biological nanoparticles and their future prospects.

Keywords: Green Nanoparticles; Biogenerators; Biosynthesis; Potential Applications; Future Prospective.

INTRODUCTION

Nanoscience and nanotechnology are two keywords considerably used this time to label wild types of research effectiveness around the world. Nanotechnology can be explained as manipulation and production of materials extended in size from one to hundred nm (Mohanpuria et al., 2008). The nanometer (nm) is a metric unit of length, and denotes one billionth of a meter or $10^{-9}$m. Collectively, nanotechnology describes as the science participate nanoscale particles that increase the scope of investigating and regulating the interplay at cell level between synthetic materials and biological systems (Du et al., 2007). It is divided to three types (Singh et al., 2008) computational nanotechnology which deals with modeling and stimulating of the complex nanometer scale structure, dry nanotechnology which deals with the surface science, physical chemistry and fabrication of structures in silicon, carbon, and inorganic materials and wet nanotechnology which deals with the biological systems such as enzymes and cellular components (Sinha et al., 2009), these three fields are interdependent to each other. A new branch of nanotechnology is nano-bio-technology...
which combines biology principles with physical and chemical procedures to generate nano-sized particles with specific properties. Nanoscale materials with at least one dimension between one and hundred nm exhibit unique electronic, optical, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials (Yang et al., 2010). Many material has constant physical properties regardless of its size, but at the nanoscale, they exhibit interesting and exclusive properties due to the fact that nanoparticles have a very high surface area to volume ratio (Thakkar et al., 2010), and this alters thermal, mechanical and catalytic properties of the material.

Green synthesis of nanoparticles
It has opened several arms in the development of new nano materials and examining their properties by tuning the particle size, shape and distribution (Bar et al., 2009; Das and Velusamy, 2014). Metal nanoparticles have been extensively studied due to their specific characteristics such as catalytic activity, optical, electronic, magnetic or antimicrobial properties (Narayanan and Sakthivel, 2010). Traditionally, aerosol technologies, laser ablation, lithography, ultrasonic fields, UV irradiation, and photochemical reduction techniques have been used successfully to produce different metal nanoparticles such as platinum, gold, silver, and palladium. However, considering the fast growth in the usage of nano-materials in diverse fields, there is an urgent need to develop clean, simple, nontoxic, and eco-friendly procedures for their formation. Particularly, synthesis of noble metal, silver and gold nanoparticles using natural organism has become a major research area in the field of nanotechnology. This may due to their simplicity of procedures, stability of nanoparticles, and their potential applications in chemical sensing, biological imaging, antimicrobial, gene silencing, drug delivery (Wei and Qian, 2008).

Several studies have reported natural polymers such as chitosan, starch and tannic acid as reducing agents for the synthesis of silver and gold nanoparticles (Dadosh, 2009; Li et al., 2011).

Silver nanoparticles
It has been positioned as the 47th element in the periodic table, having an atomic weight of 107.8 and two natural isotopes 106.90 Ag and 108.90 Ag with abundance of 52 and 48% whereas the colloidal silver is of particular interest because of distinctive properties such as good conductivity, chemical stability, catalytic and antibacterial activity (Frattini et al., 2005). The medicinal and preservative properties of silver have been known for over 2,000 years. Silver is one of the basic elements that make up our planet. It is a rare, but naturally occurring element, slightly harder than gold and very ductile and malleable. AgNPs of many different shapes (rod-shaped, spherical, truncated, triangular nanoplates) were developed by various synthetic routes. Truncated triangular silver nanoplates were found to show the strongest anti-bacterial activity. The AgNPs have excellent antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. Silver ions and nanoparticles are highly toxic and hazardous to microorganisms. AgNPs have many applications; for example, they might be used as spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for bio labelling and as antimicrobials. (Joerger et al., 2000; Magudapathy et al., 2001; Panacek et al., 2006). Currently AgNPs are wildly used as antibacterial and antifungal agents in a diverse range of consumer products: air sanitizer sprays, detergents, soaps, shampoos, toothpastes and washing machine (Buzea et al., 2007). In the synthesis of AgNPs carob leaf extract shown better speed in compared with other extracts (Akl et al., 2013). The synthesis of stable AgNPs by the bio reduction method was investigated. Aqueous extracts of the manna of hedysarum plant and the soap-root (Acanthe phylum bracteatum) plant were used as reducing and stabilizing agents (Mehrdad and Khalil, 2010).

Gold nanoparticles
Gold is a well-known biocompatible metal and colloidal gold was used as a drinkable solution that exerted curative properties for several diseases in ancient times (Daniel and Astruc, 2004). AuNPs have a great bactericidal effect on a several range of microorganisms; its bactericidal effect depends on the size and shape of the particle (Dror-Ehre et al., 2009). Recently there are a few, reports that algae is being used as a biofactory for synthesis of metallic nanoparticles (Almeida et al., 2011). AuNPs have wide range of applications in nano-scale devices and technologies due to its chemical inertness and resistance to surface oxidation (Sugunan et al., 2005). Preparation of nanoscaled gold materials
has become very important due to their unique properties, which are different from those of the bulk materials (Armendariz, 2004). AuNPs play a vital role in nanobiotechnology as biomedicine because of convenient surface bioconjugation with biomolecular probes and remarkable plasmon-resonant optical properties (Kreibig and Vollmer, 1995; Daniel and Astruc 2004; Wu and Chen 2010). Many research articles reported the synthesis of AuNPs using plant extracts such as Cinnamomum zeylanicum (Smitha et al., 2009), Mangifera indica (Philip, 2010), Banana peel (Bankar, et al., 2010), Brevibacterium casei (Kalishwaralal et al., 2010 and Mittal et al., 2013), Cochlodina gossypium (Vinod et al., 2011), Memecylon edule (Elavazhagan and Arunachalam, 2011), Nycanthes arboritris (Das et al., 2011), Murraya Koenii (Philip et al., 2011), Macrotlyloma uniflorum (Aromal et al., 2012), Terminalia chebula (Kumar et al., 2012), Ficus religiosa (Wani et al., 2013) Memecylon umbellatum (Arunachalam et al., 2013), Citrus limon, C. reticulata and C. sinensis (Sujitha and Kannan, 2013), Piper pedicellatum (Tamuly et al., 2013), Euphorbia hirta (Annamalai et al., 2013). AuNPs have an important function in the delivery of nucleic acids, proteins, gene therapy, in vivo delivery, targeting, etc (Tiwari et al., 2011). In the recent decade, gold nanoparticles (NPs) (Dyman et al., 2008) have attracted significant interest as a novel platform for various applications such as nanobiotechnology and biomedicine. Nano size gold, an emerging nanomedicine is renewed for its promising therapeutic possibility high surface reactivity, resistance to oxidation and Plasmon resonance (Guo et al., 2005).

**Biogenerators of nanoparticles**

**Algae nanoparticles**

The ability of organisms in nanoparticle synthesis has opened a new, exciting approach towards the development of these natural nanofactories. It seems that the optimization of reaction conditions can control the morphologies and other properties of nanoparticles. Therefore, researchers have focused their attention on finding optimal reaction conditions and cellular mechanisms involved in the reduction of metal ions and the formation of nanoparticles (Riddin et al., 2006; Govender et al., 2009). Rapid formation of gold nanoparticles through extracellular biosynthesis has been made feasible in a marine alga, Sargassum wightii Greville (Singaravelu et al., 2007). S. wightii was able to form high-density and extremely stable gold nanoparticles (8–12 nm) extracellularly, in a short timespan (Singaravelu et al., 2007). When Spirulina platensis biomass was exposed to 10^{-3} M aqueous AgNO_3 and HAuCl_4, extracellular formation of spherical gold (6–10 nm), silver (7–16 nm) and bimetallic Au–Ag nanoparticles (17–25 nm) resulted after 120 h at 37°C at pH 5.6 (Govindaraju et al., 2008). Proteins might be responsible for the reduction and stabilization of nanoparticles. Studies on the biosorption and bioreduction of Au(III) ions are ongoing, and Fucus vesiculosus, a brown alga, is important in this respect (Mata et al., 2009). Bioreduction with F. vesiculosus could be used as an alternative eco-friendly process for recovering Au from dilute hydrometallurgical solutions and leachates of electronic scraps. In another study, the extracellular biosynthesis of self-assembled spherical gold nanoparticles (15–20 nm) was accomplished using the aqueous extract of the brown alga Laminaria japonica. El-Rafie et al. (2012) reported the synthesis of silver nanoparticles using polysaccharides extracted from four marine macroalgae, namely, Pterocladium capililaceae, Jania rubens, Ulva fasciata and Colpomenia sinuosa, as reducing agents for silver ions, as well as stabilizing agents for synthesized silver nanoparticles. Hot water extracts of the algae P. capililaceae, J. rubens, U. fasciata and C. sinuosa, were studied for their polysaccharide content and were found to be 6.46, 5.63, 8.84 and 4.33%, respectively. GLC analysis of these extracted polysaccharides revealed that rhamnose (46.88% in P. capililaceae), galactose (30.2% in U. fasciata; 22.23% in J. rubens) and fucose (10% in C. sinuosa) constituted the major sugars, comprising part of a structural polysaccharide. In another study, the synthesis of silver nanoparticles (13 ± 3 nm) through a green route using sulfated polysaccharide isolated from marine red algae (Porphyra vietnamensis) was reported (Venkatpurwar and Pokharkar, 2011). Extracellular biosynthesis of gold nanoparticles using Padina gymnospora has been attempted and rapid formation of gold nanoparticles was achieved in a short time frame (Singh et al., 2013). AFM analysis showed the results of particle sizes (53–67 nm) and average height of particle roughness (60 nm). Participation of the polysaccharides of the algal cell wall in the reduction process was confirmed by FTIR analysis of the biomass after gold recovery.
Fungal nanoparticles

Fungi are a relatively recent addition to the list of microorganisms used for nanoparticle synthesis. They grow in the form of mycelial mesh which helps them to bear flow pressure and agitation and other conditions to which microbes are subjected to in a bioreactor used for large scale production. Though they require better precision and care to grow but they are easier to handle and manipulate. The nanoparticles are generally generated extracellularly (sometimes intracellular production does occur) they are devoid of various impurities from the cell and can be used directly (Mohammadian et al., 2007).

The use of eukaryotic organisms for nanoparticle synthesis was first demonstrated by use of Verticillium sp. for the synthesis of gold nanoparticles. In this experiment, gold nanoparticles were reported on the surface and cytoplasmic membrane of the fungal mycelia. Due to the formation of gold nanoparticles the mycelial mass attains a typical purple color demonstrating the formation of gold nanoparticles the mycelial network. TEM analysis shows that particles of well-defined geometry like triangular, hexagonal or spherical shape were formed on the cell wall and quasi-hexagonal morphology were formed of the cytoplasmic membrane. The fungal biomass on exposure to silver nitrate solution was also found generate silver nanoparticles intracellularly. The powder diffraction indicates the crystal nature of both the nanoparticles. The exact mechanism for the synthesis of nanoparticles by Verticillium is not yet known. It is thought that the first step is the interaction between positively charged metal ions and negatively charged carboxylates on the enzymes present in fungal cell wall and adhesion of the metal ions to the surface as a result of this interaction. The enzymes reduce these metal ions to elemental metal which serve as nucleation sites and further growth is carried out by subsequent reduction and accumulation. The ability of Verticillium to grow and replicate even after exposure to metal ions demonstrate their ability to be used commercially for production of nanoparticles (He et al., 2007).

A plant pathogenic fungus, Fusarium oxysporum has also been studied extensively. It

<table>
<thead>
<tr>
<th>Algae</th>
<th>Nanoparticle</th>
<th>Size (nm)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>Cadmium sulfide</td>
<td>-</td>
<td>Hu et al., 2001</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>Gold</td>
<td>-</td>
<td>Mandal et al., 2006</td>
</tr>
<tr>
<td>Gelidiella acerosa</td>
<td>Silver</td>
<td>22</td>
<td>Vivek et al., 2011</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>Cadmium sulfide</td>
<td>-</td>
<td>Mandal et al., 2006</td>
</tr>
<tr>
<td>Sargussum wightii</td>
<td>Gold</td>
<td>8-12</td>
<td>Singaravelu et al., 2007</td>
</tr>
<tr>
<td>Spirulina platensis</td>
<td>Silver</td>
<td>7-16</td>
<td>Govindaraju et al., 2008</td>
</tr>
<tr>
<td>Spirulina platensis</td>
<td>Gold</td>
<td>6-10</td>
<td>Govindaraju et al., 2008</td>
</tr>
<tr>
<td>Tetraselmis kochinensis</td>
<td>Gold</td>
<td>17-25</td>
<td>Govindaraju et al., 2008</td>
</tr>
<tr>
<td>Tetraselmis suecica</td>
<td>Gold</td>
<td>5-35</td>
<td>Senapati et al., 2012</td>
</tr>
<tr>
<td>Acanthophora specifera</td>
<td>Silver</td>
<td>33-81</td>
<td>Ibraheem et al., 2016</td>
</tr>
<tr>
<td>Parachlorella kessleri SAG 211-11</td>
<td>AgCl</td>
<td>10.3–47</td>
<td>Hamed et al., 2016</td>
</tr>
<tr>
<td>Anabaena variabilis</td>
<td>Silver</td>
<td>12-20</td>
<td>Hamed et al., 2017</td>
</tr>
<tr>
<td>Galaxaura elongate</td>
<td>Gold</td>
<td>3.85–77.13</td>
<td>Abdel-Raouf et al., 2017</td>
</tr>
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<td>Laurencia catarinensis</td>
<td>Silver</td>
<td>39.41–77.71</td>
<td>Abdel-Raouf et al., 2018</td>
</tr>
<tr>
<td>Padina pavonia</td>
<td>Silver</td>
<td>49.58–86.37</td>
<td>Abdel-Raouf et al., 2018</td>
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Algal pigments, such as fucoxanthins, a type of carotenoid rich in hydroxyl groups, could also have participated in the gold reduction. These pigments have reductive properties and are released to solution by diffusion. These soluble elements could have acted as capping agents, preventing the aggregation of nanoparticles in solution and playing a relevant role in their extracellular synthesis and shaping. For instance, the pigment C-phycoerythrin (C-PE), extracted from the marine cyanobacterium Phormidium tenue NTDM05, was used to synthesize CdS nanoparticles (about 5 nm). Essentially, it was found that the pigment stabilized the CdS nanoparticles (Mubarak Ali et al., 2012). Synthesis of nanoparticles by algae is more environmentally friendly than purely chemical synthesis. However, the choice of algae is very important. In Table 1, important examples of nanoparticle biosynthesis using algae are listed.
was found that it was able to generate gold and silver nanoparticles extracellularly rapidly. This was observed from the fact that the supernatant changed its color but the mycelia mass retained its original color. Moreover the fungal extract was also able to generate gold and silver nanoparticles. It is believed that the fungus releases reductases in the solution which are responsible for the reduction of metal ions. This makes in-vitro generation of nanoparticles using an enzyme/cell extract based process possible. It was recently discovered that when *F. oxysporum* is exposed to equimolar solutions of hydrogen tetrachloroaurate (III) and silver nitrate led to the production of gold-silver alloy. The presence of only one plasmon resonance, shifting gradually from gold to silver and back indicates the formation of homogeneous alloy rather than segregated metal or core/shell type structure. The fungus on exposure to cadmium sulfate solution was found to yield cadmium sulfide quantum dots (5-20 nanometers) with hexagonal morphology. Long term incubation of the fungus with cadmium nitrate does not yield cadmium sulfide nanoparticles which indicate the action of sulfate reducing enzyme. Polyacrylamide gel electrophoresis of the extract led to four different protein bands. These proteins were extracted using dialysis and addition of ions to this solution does not yield cadmium sulfide which attests to the presence of some other factor. Addition of ATP and NADH restored the capability to produce quantum dots (Gercke and Pinches, 2006).

Yeast are eukaryotic microorganisms and generally, several strains play an important role in food industry due their ability to sugar fermentation. A novel approach of their use could be a production of metal nanoparticles and nanostructures via their reducing enzymes intracellularly or extracellularly. The focus of this review is the application of yeast in the green synthesis of inorganic nanoparticles and the innovation use in the fermentation industry (Skalickova et al., 2017). Yeasts are most useful in the synthesis of semiconductor nanoparticles like cadmium sulfide, lead sulfide, antimony oxide, etc. *Torulopsis sp.* was the first yeast in which synthesis of face centered cubic structured PbS nano-crystals, showing semiconductor properties, which was intracellularly produced in the vacuoles having a dimension of 2–5 nm in spherical structure when incubated with Pb²⁺ of 330 nm in UV–Vis spectrophotometer. Diode junction can be formed using these nanoparticles. *S. cerevisiae* (baker’s yeast) can reduce Au³⁺ to give gold nanoparticles. Reduction happens in the peptidoglycan layer of the cell wall by the aldehyde group present in reducing sugars (Agnihotri et al., 2009; Kouzegaran and Farhadi, 2017).

Gold nanoparticles are also synthesized by the tropical marine yeast *Yarrowia lipolytica* by reducing the gold ions using pH control regulations. It synthesizes nanoparticles of various morphologies when subjected to different pH cultures. At pH 2.0 it produce hexagonal and triangular gold crystals because of the nucleation of the nanoparticles on the cell surfaces giving rise to golden color which falls in the visible range spectrum having wavelength 540 nm and at pH 7.0 and pH 9.0 gold nanoparticles gives pink and purple colors with an average size of ~ 15 nm. *S. cerevisiae* can also synthesize face-centered cubic unit cell antimony oxide (Sb₂O₃) nanoparticles with spherical morphology of size 2-10 nm at room temperature conditions. This was possibly due to the radial tautomerization of membrane-bound quinines or by membrane-bound or cytosolic pH-dependent oxidoreductases. Antimony oxides are an important ingredient for semiconductor industry. MKY3 is the only yeast so far discovered which is capable of extracellular silver nanoparticles synthesis of hexagonal crystal structure of size 2-5 nm in log phase growth of the yeast. These silver nanoparticles are used to make silver tolerant strain (Jha et al., 2009; Feng et al., 2017).

**Actinomycete nanoparticles**

Actinomycetes are the group of gram-positive filamentous bacteria which are primarily recognized as organism of academic curiosity, potential degraders and also as a potential source for antibiotics (Balagurunathan and Radhakrishnan, 2007). Although actinomycetes are well exploited for antibiotics and other high value metabolites, they are less exploited in terms of nanoparticles. The biosynthesis methods are commonly used for any compound production from microorganisms, which may yield good amount in terms of quantity. Limited reports are available on the extracellular and intracellular biosynthesis of nanoparticles by the actinomycetes (Balagurunathan et al., 2011). Synthesize many different metabolites, antibioticics represent the most known product which used in veterinary and pharmaceutical side (Shrivastava et al., 2007) .*Streptomyces* sp. positive for gram stain, filament bacteria and produce different compound with having
biologically active compounds such as hydrolytic enzymes, antibiotics, and enzyme inhibitors (Sastry et al., 2003). A biologically synthesized AgNPs using Streptomyces sp. VITBT7 isolated from soil was recorded (Williams and Cross 1971). These AgNPs showed SPR peak at 420 nm and spherical shape with 20–70 nm in size. AgNPs showed antmicrobial activity against fungal and bacterial pathogens (Subashini et al., 2013). An extracellular synthesizes AgNPs by Streptomyces sp. JAR isolated from the soil samples and with size AgNPs was 68.13 nm, and showed antimicrobial activity against bacterial and fungal pathogens (Deeppa et al., 2013).

A novel extremophilic actinomycete, Thermomonospora sp. was found to synthesis extracellular monodispersed spherical gold nanoparticles at an average size of 8 nm (Ahmad et al., 2003a). FTIR analysis confirmed the presence of amide (I) and (II) bands of protein as capping and stabilizing agent on the surface of nanoparticles. These particles were stable for more than 6 months. Electrophoretic analysis showed that the proteins of molecular weight ranged from 80 kDa to 10 kDa were involved in the stabilization of nanoparticles. In contrast, an alkalotolerant actinomycete, Rhodococcus sp. intracellularly accumulated gold nanoparticles with a dimension of 5–15 nm. The availability of reductases on the cell wall reduced Au$^{3+}$ and accumulated as Au$^{0}$ on the cell wall and on cytoplasmic membrane with good dispersity (Ahmad et al., 2003b). Although actinomycetes are regarded as the primary sources for the synthesis of secondary metabolites till date, these new discoveries would take a lead in further screening of actinomycetes for the synthesis of nanomaterials.

**Bacterial nanoparticles**

One approach that shows immense potential is based on the biosynthesis of NPs using bacteria (a kind of bottom up approach) (Korbekandi et al., 2009; Iravani, 2011; Iravani et al., 2014). The objects of recent studies tend to provide a controlled and up-scalable process for biosynthesis of monodispersed and highly stable NPs. Thus, a wide number of bacterial species have been used in green nanotechnology to research alternative methods for the synthesis of NPs. Researchers have started to use biomass or cell extracts of bacteria for synthesizing NPs. Bacteria are considered as a potential biofactory for the synthesis of NPs like gold, silver, platinum, palladium, titanium, titanium dioxide, magnetite, cadmium sulphide, and so forth. Some well-known examples of bacteria synthesizing inorganic materials include magnetotactic bacteria and S layer bacteria. Most metal ions are toxic for bacteria, and, therefore, the bioreduction of ions or the formation of water insoluble complexes is a defense mechanism developed by the bacteria to overcome such toxicity (He et al., 2007; Lengke et al., 2007). Cell-free culture supernatants of five psychrophilic bacteria Phaeocystis antarctica, Pseudomonas proteolytica, Pseudomonas meridiana, Arthrobacter kerguelensis, and Arthrobacter gangotriensis and two mesophilic bacteria Bacillus indicus and Bacillus cecembensis have been used to biosynthesize silver NPs (6–13 nm). These NPs were stable for 8 months in the dark. The synthesis and stability of silver NPs appeared to depend on the temperature, pH, or the species of bacteria from which the supernatant was used. It was observed that the A. kerguelensis supernatant could not produce silver NPs at the temperature where P. antarctica could synthesize silver NPs (Shivaji et al., 2011).

The rapid biosynthesis of silver NPs using the bioreduction of aqueous Ag$^{+}$ ion by the culture supernatants of Klebsiella pneumonia, E. coli, and Enterobacter cloacae (Enterobacteriaceae) was reported (Shahverdi et al., 2007). The synthetic process was quite fast and the produced NPs were formed within 5 min of silver ions coming in contact with the cell filtrate. Piperitone (a natural product which show an inhibitory effect on nitro reduction activity of Enterobacteriaceae) could partially inhibit the bioreduction of silver ions to silver NPs by different strains of Enterobacteriaceae including K. pneumoniae. As a result of this control experiment, nitroreductase enzymes might be responsible for bioreduction of silver ions. Recently, it was shown that visible-light emission could significantly prompt synthesis of silver NPs by culture supernatants of K. pneumonia (Mokhtari et al., 2009). Silver NPs with uniform size and shape (1–6 nm) were biosynthesized using silver chloride (as the substrate). When some quantities of NaOH were added to the bioreduction system, silver NPs were successfully prepared with reduction of (Ag(NH$_3$)$_2$)$_2$+ by Aeromonas sp. SH10 and Corynebacterium sp. SH09 (Fu et al., 2006). It was speculated that (Ag(NH$_3$)$_2$)$_2$+ first reacted with OH$^-$ to form Ag$_2$O, which was then metabolized independently and reduced to silver NPs by the biomass. The color of the bioreduction system changed from pale yellow to dark yellow. The silver NPs were...
monodispersed and uniform in size without distinct aggregation and it was observed that the solution containing the NPs remained stable for more than six months. The color change indicated the formation of silver NPs in the reaction mixture, as it is well-known that silver NPs exhibit striking colors (light yellow to brown) due to excitation of surface plasmon vibrations (SPV) in the particles (Kapoor, 1998).

It was reported that *Bacillus subtilis* 168 was able to reduce Au\(^{3+}\) ions to octahedral gold NPs (5–25 nm) within bacterial cells by incubation of the cells with gold chloride under ambient temperature and pressure conditions (Beveridge and Murray, 1980; Southam and Beveridge, 1994). The reduction processes of chloroaurate and silver ions by *B. subtilis* were found to be different (Satyanarayana et al., 2010). Gold NPs were biosynthesized both intracellularly and extracellularly, while silver NPs were exclusively formed extracellularly. The gold NPs were formed after one day of addition of chloroaurate ions, while the silver NPs were formed after seven days. Transmission electron microscopy (TEM) micrographs depicted the formation of gold NPs intracellularly and extracellularly, which had an average size of 7.6±1.8 and 7.3± 2.3 nm, respectively, while silver NPs were exclusively formed extracellularly, with an average size of 6.1 ± 1.6 nm. The bacterial proteins were analyzed by sodium dodecyl sulfonatepolyacrylamide electrophoresis (SDS-PAGE) before and after the addition of metal ion solutions. Satyanarayana et al., (2010) reported that proteins of a molecular weight between 25 and 66 kDa could be responsible for chloroaurate ions reduction, while the formation of silver NPs could be attributed to proteins of a molecular weight between 66 and 116 kDa. They reported that the NPs were stabilized by the surface-active molecules, that is, surfactin or other biomolecules released into the solution by *B. subtilis*.

**Viral nanoparticles**

Over the past decade, there has been an increased interest in the biomaterials field for various applications. The development of cancer treatment methods (chemotherapy, radiation and hormone therapy) has saved millions of lives. However, the problem with this treatment remains the side effects (weakness, nausea, falling hair, and flu-like symptoms). Additionally, because this treatment doesn’t differentiate between healthy and cancerous cells due to the low efficacy of such drugs. This was the driving force for many scientists and researchers to engineer and design alternative drug delivery systems. One such valuable endeavor is the development of virus-based platforms for the targeted drug delivery. Viral nanoparticles offer unmatched monodispersity platform in comparison to the synthetic nanoparticles. In addition, they are biocompatible, can be produced in large quantities and very cheap make them ideal nanoparticles. Various classes, shapes and materials composition have been reported in the literature for molecular targeting purposes. The focus of researchers in this field is in the development of plant-virus based nanoparticles to minimize the chance of the virus being infectious or causing disease to the individuals. Virus propagation is supported by the suppressive and permissive cells to cause infection. Therefore, developing plant virus drug delivery system promises to minimize the toxic side effects of the chemotherapeutics by delivering selectively to the targeted cells, reducing the immune response and interacting with mammalian cells (Aljabali, 2017).

Over the past few years, plant viruses have found a novel role as nanoparticles, to not only deliver medical materials such as drugs, but also sources of material for electronics and optics, as examples (Narayan and Han, 2017). It is striking to note that several plant viruses have been shown to accumulate at solid tumors and elicit a highly localized immune response within the surrounding microenvironment (Peruzzi and Chiocca, 2016). As plant virus nanoparticle systems improve in sophistication, they are being examined for their ability to activate the T cell response, and thus represent alternative immunotherapy strategies for cancer and other chronic diseases (Peruzzi and Chiocca, 2016).

Nanoparticles based on plant viruses do not cause adverse reactions, and yet can be highly immunogenic, they have so far only been tested on preclinical models. These characteristics make plant virus nanoparticles prospective vehicles for use in developing countries where patients have low access to modern medical treatment and thus poor prognosis for survival. Plant viruses are frequently characterized by their icosahedral or helical morphologies; these differences offer a range of attributes which make them suitable for a variety of purposes in medicine as well as in engineering (Czapar and Steinmetz, 2017). Both morphologies can carry drugs or imaging reagents that are conjugated to the outer surface of the capsid protein, thus producing a highly regular repeating pattern on the surface of the virus.
particle (Narayanan and Han, 2017). The helical shape of some plant viruses appears to assist them in their ability to home in on solid tumors (Narayanan and Han, 2017). Icosahedral viruses, on the other hand, can carry reactive molecules such as drugs within their internal cavity and release them under the appropriate physiological conditions (Lico et al., 2012; Lico et al., 2015).

**Plant nanoparticles**

The use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, eco-friendly, non-pathogenic, economical protocol and providing a single step technique for the biosynthetic processes (Ahmed, et al., 2016). The reduction and stabilization of silver ions by combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpinoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures (Kulkarni and Muddapar, 2014). The protocol for the nanoparticle syntheses involves: the collection of the part of plant of interest from the available sites was done and then it was washed thoroughly twice/thrice with tap water to remove both epiphytes and necrotic plants; followed with sterile distilled water to remove associated debris if any. These; clean and fresh sources are shade-dried for 10–15 days and then powdered using domestic blender. For the plant broth preparation, around 10 g of the dried powder is boiled with 100 mL of deionized distilled water (hot percolation method). The resulting infusion is then filtered thoroughly until no insoluble material appeared in the broth. To 10^{-3} M AgNO_{3} solution, on addition of few mL of plant extract follow the reduction of pure Ag(I) ions to Ag(0) which can be monitored by measuring the UV–visible spectra of the solution at regular intervals (Sahayaraj and Rajesh, 2011).

The relatively high levels of the steroids, sapogenins, carbohydrates and flavonoids act as reducing agents and phyto-constituents as the capping agents which provide stability to silver nanoparticles. The synthesized nanoparticles found to be of average size around 7–17 nm and are of spherical shaped. These nanoparticles were found to have a crystalline structure with face centered cubic geometry as studied by XRD method. By using tea as a capping agent, 20–90 nm silver nanoparticles were synthesized with crystalline structure. Reaction temperature and the dosage of the tea extract showed an effect on the production efficiency and formation rate of nanoparticles (Suna et al., 2014). The size of spherical shaped silver nanoparticles is ranging from 5 to 20 nm, as evident by TEM. With increasing intensity of extract during the period of incubation, silver nanoparticles showed gradual change in colour of the extracts to yellowish brown with callus extract of the salt marsh plant, *Sesuvium portulacastrum* L (Nabikhan, et al., 2010).

Silver nanoparticles were synthesized on reduction of silver nitrate solution by aqueous extract of *Azadirachta indica* leaves by some workers (Ramya and Subapriya, 2012). and the growth kinetics of silver nanoparticles was investigated having size of 10–35 nm. Colloidal silver nanoparticles were synthesized by an easy green method using thermal treatment of aqueous solutions of silver nitrate and natural rubber latex extracted from *Hevea brasiliensis*. The silver nanoparticles presented diameter ranging from 2 nm to 10 nm and had spherical shape with face centred cubic (fcc) crystalline structure (Ramya and Subapriya, 2012).

**Mechanism of nanoparticles synthesis**

Though different biogenic sources have been identified for synthesis of nanoparticles, the mechanism of synthesis is yet to be deciphered clearly. Many theories have been suggested to explain the mechanism of synthesis, especially for metal nanoparticles. As biogenic metal nanoparticles have been found to be stable for three months and more, it is evident that there should be a reducing agent as well as a stabilizing agent in the system that would facilitate formation and stabilization of the metal nanoparticles. The identification of these components from the complex cellular environment is extremely challenging. The nature of the reducing agent and stabilizing agent also varies with the species employed for the synthesis. In general, peptides, proteins, polyols and heterocyclic compounds have been suggested to have an important role in the synthesis of metal nanoparticles through an electron shuttle or charge capping mechanism. Peptides have been investigated for their role as possible reducing agents as well as stabilizing agents in many microbial species. Interestingly, several reports have indicated that a single amino acid might not be as effective as a polypeptide sequence containing the same amino acid residue for the synthesis. The metal ion is first reduced to form metal nanoparticle, which acts as a nucleus.
for further growth of the metal crystal. The peptide is initially thought to adsorb to the surface of the metal nanoparticle clusters causing a localized reducing environment that results in the reduction of more metal ions at the interface between the peptide and the metal nuclei. As a result, nanoparticles with large size distributions are formed.

**Silver nanoparticles: Mechanism of synthesis**

**Enzymatic synthesis:**

Many mechanisms involving many biomolecules have been proposed for the biosynthesis of nanoparticles, but the most accepted mechanism involves nitrate reductase-dependent reduction of silver ions (Anil Kumar et al., 2007; Kalimuthu et al., 2008). Nitrate reductase is an enzyme that is co-factored by nicotinamide adenine dinucleotide (NADH) (Anil Kumar et al., 2007) and is capable of reducing silver ions to silver nanoparticles. Basically, it is an enzyme that is responsible for the conversion of nitrate in the nitrate cycle (Durán et al., 2005). This mechanism has been clearly studied in B. licheniformis (Kalimuthu et al., 2008), as it secretes many NADH-dependent enzymes in which the effect of a-NADH dependent nitrate reductase on silver ions also has been clearly studied. The enzyme nitrate reductase converts nitrate to nitrite and will transfer an electron to silver ions (Ag+) to form free silver (Ag0). However, the synthesis would take only at lower concentrations of silver nitrate: at higher concentration, it would lead to cell destruction (Kalimuthu et al., 2008; Pandian et al., 2010). The synthesis of nanoparticles could be considered rather as a defense mechanism by organisms against silver ions. Silver ions, due to their antimicrobial activity, could be harmful to these organisms. Hence, these organisms develop some mechanisms to protect them by neutralizing the ions. Silver ions can exhibit their antimicrobial activity in many ways. They bind to the negatively charged DNA molecules, altering their structure, which inhibits DNA replication. Free silver ions bind to the thiol containing protein groups and inhibit their function and, most importantly, they produce reactive oxygen species, thus forming highly reactive radicals like hydrogen peroxide, which can destroy the cell. These radicals are produced in the respiratory system when the NADH dehydrogenase is inhibited by silver ions (Gautam and Sharma, 2002; Matsumura et al., 2003). These highly reactive oxygen species are found to have degenerative effects on the cells. This could be neutralized by catalase, which would catabolize those reactive oxygen species. In B. licheniformis, the increase in silver ion concentration is accompanied by an increase in catalase synthesis, and when the silver ion concentration increases beyond the threshold level, the catalases cannot help the cell to survive. Thus, apoptosis could be induced at higher levels of silver ions. In order to prevent this, silver ions are converted to their inactive silver by their defense mechanisms (Anthony et al., 2013).

**Peptide-mediated synthesis:**

The use of peptides for the biogenesis of nanoparticles was first shown by Naik and coworkers. The peptides with reducing amino acid moieties like arginine, cysteine, lysine and methionine can readily reduce metal ions (Naik et al., 2002). These peptides bind over the silver clusters and facilitate easy reduction of ions over the surfaces to build up silver nanoparticles. The amino acid tyrosine acts as a reducing agent at high pH and assists the reduction of silver ions by ionizing the phenolic groups in tyrosine (Selvakannan et al., 2004).

**Gold nanoparticles: Mechanism of synthesis**

**Enzymatic synthesis:**

A model for enzymatic gold nanoparticle synthesis and stabilization in Stenotrophomonas maltophilia was proposed by Nangia et al., (2009). The enzyme involved is NADPH-dependent reductase, which reduces Au3+ to Au0. The peak value of zeta potential for gold nanoparticles is –16.7 mV. Hence, it has to be capped by negatively charged groups, and the phosphate ions of NADP serve this purpose (Nangia et al., 2009). Extracellular biosynthesis and characterization of gold nanoparticles was made in Rhizopus oryzae (Das et al., 2009). After the addition of AuCl4 - in fungal culture, amide I, II and III groups appeared, along with loss of carboxyl groups in the mycelium, thus showing the importance of polypeptides/proteins in the formation of gold nanoparticles. Also, in spectral analysis, shifts in infrared peaks indicated the involvement of phosphate bonds in the reduction. Thus, this shows that surface-bound protein molecules are important factors in gold nanoparticle synthesis (Das et al., 2009).

**Biотemplate-mediated synthesis:**

As mentioned earlier, viral capsid can be used as
a template for gold nanoparticle synthesis. The active tyrosine residues on the potentiated viral capsid surface reduce AuCl₄⁻ and form gold nanoparticles on the surface. This reduction is enhanced by incorporating tyrosine sites within the capsid cavity or surfaces. Furthermore, the ratio of tyrosine to histidine residues can be varied to enhance gold nanoparticle production (Sločik et al., 2005). It was also revealed that tyrosine-containing oligopeptides could only synthesize nanoparticles, thus showing the importance of reducing amino acids for nanoparticle synthesis (Ray et al., 2006).

### Applications of nanoparticles:

Applications Nowadays, biosynthesized nanoparticles, especially gold and silver, were used in diverse applications especially in diagnostic field due to its antibacterial, antifungal, larvicidal, antifouling, anticancerous, antioxidant properties. Green synthesis of nanoparticles using dextran as ligand was explored since dextran was considered as cheaper, nontoxic and biocompatible agent (Deepa et al., 2013). (a)

### Antibacterial activity:

Using *Streptomyces viridogens*, gold nanoparticles of spherical shape with 18-20 nm was synthesized through intracellular mode (Ahmad et al., 2003b). A considerable number of silver nanoparticles using *Streptomyces* sp. were reported to show antibacterial activity against varied human pathogens through extracellular mode (Singh, 2016). Unnamed actinomycetes mediated silver nanoparticles were also reported to produce antibacterial activity (Priyaragini et al., 2013). Rare actinobacteria such as *Thermoactinomyces* sp. produce spherical shaped 20-40 nm sized silver nanoparticles were reported by Deepa et al. (Zarina and Nada, 2014). Similarly, Shanmugasundaram et al., (2013) reported the production of copper and zinc nanoparticles sized 100-150 nm using *Streptomyces* sp. showing antimicrobial activity. The mechanism behind this bactericidal effect is silver nanoparticles attach to the bacterial cell membrane and disturb both respiration and permeability. Then by penetrating deep into the DNA part, it causes further damage by disrupting DNA replication due to release of silver ions, which leads to target cell destruction (Hussain et al., 2014). (b)

### Antifungal activity:

Biosynthesis of gold nanoparticles using *Streptomyces* sp. VITDDK3 showed antifungal activity against *M. gypseum* and *T. rubrum* by changing the membrane potential and inhibiting ATP synthase activity of the target cell (Oza et al., 2012). (c)

### Anti-biofouling property:

anti-biofouling is defined as the process of eliminating the microbes that aggregate on wetted surface forming biofilms leading to foul smell production. Alani et al., (2012) reported the antibiofouling property of 5-50 nm spherical-shaped silver nanoparticles using *Streptomyces naganishii* MA7. (d)

### Antioxidant property:

an antioxidant prevents the oxidation of other molecules by interfering with defense mechanism leading to ROS generation. Antioxidant property of silver nanoparticles mediated by *Streptomyces naganishii* MA7 was reported by Alani et al., (2012). It was showing positive DPPH scavenging activity. (e)

### Larvicidal property:

Silver nanoparticles synthesized by using *Streptomyces* sp. GRD showed effective larvicidal activity against *Culex quinquefasciatus* and *Aedes aegypti*, which would be an effective bioprocess for mosquito control (Kaler et al., 2010). Nanoparticles penetrate through larval membrane into the intracellular space leading to denaturation of organelles and enzymes (Kaler et al., 2010). They reported the biosynthesis of gold nanoparticles with antimalarial activity using *Streptomyces* sp. LK3 (Ahmad et al., 2003a). (f)

### Anticancerous property:

Varied researches are going on in order to treat cancer and reduce its side effects worldwide. Manivasagan et al. reported the cytotoxic effect of silver nanoparticles mediated by *Nocardiopsis* sp. MBRC-1 (Manivasagan et al., 2013). Same way, an actinomycete PSBVIT-13 (Priyaragini et al., 2013) and *Streptomyces naganishii* MA7 (Alani et al., 2012) were also reported to mediate silver nanoparticles with cytotoxic effect. (g) Gold Nanoparticles for the Treatment of Cancer, Cancer being killer disease researchers is looking for a cure to treat it. Cancer cells along with other organisms such as bacteria, viruses and DNA can be damaged by a technique like nanophotothermalysis with lasers and gold Nanoparticles. Gold Nanoparticles are probes for application in biomedical field because they can...
be easily prepared unlike other fluorescent probes (cancer) it is an invasive because one cell left over can lead to the regrow. The nanoparticles will create bubbles in contact with cell due to extreme temperature change. These bubbles burst sending out the shockwaves which damages and causes them to lyse. This technique allows selecting only the cancer cells or abnormal cells. However this technique has less efficiency in case of solid tumor, bones, and therosclerotic plaques (Letullin et al., 2006). Accordingly to Letullin et al., (2006) the altitude of this technique has reached due to laser wavelength, pulse duration, particle size and particle shape. Thus this technique leads to the increase in life span of human lives. (h)

**Nanodiagnostics:**
Nanodiagnostics devices can be used for early disease identification at the cellular and molecular levels. Nanomedicine could increase the efficiency and reliability of in vitro diagnostics, through the use of selective nanodevices to collect human fluids or tissue samples and to make multiple analyses at the sub cellular level. From an in vivo perspective, nanodevices might be inserted into the body to identify the early presence of a disease, or to identify and quantify toxic molecules, tumor cells, and so forth (Freitas, 1999). (i)

**Nanoparticles in stem cell therapy:**
Nanoparticles have advantages over today’s therapy because to have certain properties or to behave in certain way. Nanoparticles have been used successfully by chemical engineers to enhance stem cells ability to regenerate the damaged vascular tissue and reduced muscle degeneration in mice. This allows the use of nanoparticles in gels, paints, polymers composites and high tech foams, living cells (Sagadevan et al., 2016).

**Future prospects**
There is much debate on the future implications of nanobiotechnology. It could create and suggest implementation of a choice of various new materials and devices potentially useful in the field of medicine, electronics, biomaterials and energy production. Nevertheless, this approach raises many of the same issues as any new technology, including problems with toxicity and environmental impact of nano materials (Buzea et al., 2007) and their potential effects on global economics, as well as speculation about various doomsday scenarios. These concerns have accounted for a debate among advocacy groups and governments on whether special statutory regulation of nanobiotechnology is warranted.

Despite the existence of some disputes, this technology renders immense hope for the future. It may lead to innovations by playing a prominent role in various biomedical applications ranging from drug delivery and gene therapy to molecular imaging, biomarkers and biosensors. One of these applications being the prime research objective of the present time would be target-specific drug therapy and methods for early diagnosis and treatment of diseases (Sahoo and Labhasetwar, 2003). Two types of medical applications are already emerging, both in clinical diagnosis and in R&D. Imaging applications, such as quantum dot technology are already being licensed and applications for monitoring cellular activities in tissue are coming soon. The second major type of application involves the development of highly specific and sensitive means of detecting nucleic acids and proteins (Milunovich and Roy, 2001). Continuous advancements in nanomedicine have opened up its opportunities for application in a variety of medical disciplines. Its future application as diagnostic and regenerative medicine is currently being investigated. In diagnosis, detection of diseased cells would be faster, possibly at the point of a single sick cell, while allowing diseased cells to be cured at once before they spread into and affect other parts of the body. Also, individuals suffering from major traumatic injuries or impaired organ functions could benefit from the use of nano medicine

**CONCLUSION**
Bio-based approaches are still in the development stages, and stability and aggregation of the biosynthesized NPs, control of crystal growth, shape, size, and size distribution are the most important experienced problems. Furthermore, biologically synthesized NPs in comparison with chemically synthesized ones are more polydisperse. The properties of NPs can be controlled by optimization of important parameters which control the growth condition of organisms, cellular activities, and enzymatic processes (optimization of growth and reaction conditions). Mechanistic aspects have not been clearly and deeply described and discussed. Thus, more elaborated studies are needed to know the exact mechanisms of reaction and identify the enzymes and proteins which involve nanoparticle biosynthesis. The large-scale synthesis of NPs
using different organisms is interesting because it does not need any hazardous, toxic, and expensive chemical materials for synthesis and stabilization processes. It seems that by optimizing the reaction conditions and selecting the best organism, these natural nanofactories can be used in the synthesis of stable NPs with well-defined sizes, morphologies, and compositions.

CONFLICT OF INTEREST
The authors declared that present study was performed in absence of any conflict of interest.

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