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Functional Nanomaterials Engineered by Microorganisms

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Introduction

The investigation of functional nanostructure has been widely heralded as ushering of a “new era”. Considerable efforts have been made to manufacture new nano-materials with required properties for different applications. In general, the preparation of nanostructure is achieved via either physical or chemical routes. However, the production expense of physical procedures for the synthesis of nanostructure is not cheap. Physical methods consume enormous energy due to the high temperature and pressure in the synthesis procedures. Chemical methods are lower in cost, but their disadvantages include use of toxic solvents, contamination from precursor chemicals and generation of hazardous by-products which are not environmental friendly. Recently, the development of interdisciplinary research between microbiological and material science offers an opportunity to fabricate complex hybrid nanostructures by using “living” microorganisms. For instance, in 2009, Professor Xiurong Yang’s group noticed that some features (biological behavior, chemical heterogeneity, and physical structure) of biofilm might be exploited for the generation of hierarchical structured hybrid nano-materials with some remarkable performance [1]. As a practical example, they utilized *Staphylococcus aureus* to organize ZnO/Ag bilayer “nano-filter” with impressive cell/bacteria recognition ability. In 2010, Professor David Levy’s group further extended the usage of biofilm to create electro-optical nano-device [2]. One year later, the potential of microorganism engineered nano-materials in energy research area was reported. Microorganism engineered nanostructures have already become a remarkable force behind the development of modern nanotechnology. Many researchers realized that these tiny creatures can be an efficient and low-cost avenue for the synthesis of high quality nano-devices. In this chapter, a systematic introduction is given to the history and progress of functional nanomaterials engineered by microorganisms. Particular attention is devoted to bacteria mediated nanomaterials with promising biophysical, biochemical and biomedical characteristics.

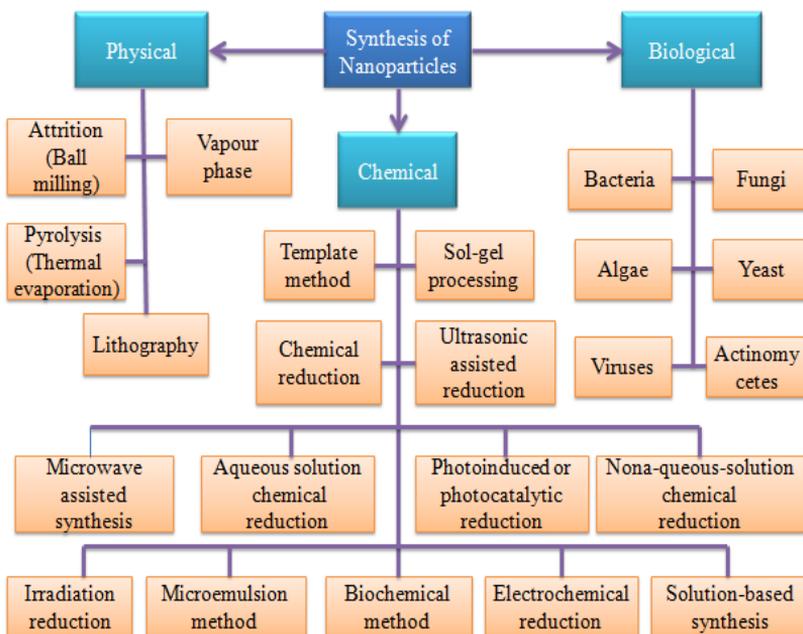


FIGURE 13.1
Different methods for synthesis of nanoparticles

History of nanostructure synthesized by microorganisms

In 1980, Beveridge and his partner studied the synthesis of Au on *Bacillus subtilis*, but it did not have much impact [3]. In 1989, Dameron's group noticed that the *Candida glabrata* could intracellularly synthesize nano-sized CdS [4]. In 1993, Edward DeLong's group realized the synthesis of magnetic nanoparticles in *magnetotactic bacteria* [5]. In 1999, Klaus's group compounded Ag nanoparticles in *Pseudomonas stutzeri* AG 259, the biosynthesis of nanomaterials started to get attention [6]. In 2001, Mukherjee's group utilized *Fusarium oxysporum* to synthesize intracellular Ag nanoparticles successfully. Furthermore, the concept of biosynthesis nanotechnology was put forward for the first time [7]. Subsequently, the biosynthesis of nanotechnology attracted widespread attention, more and more research work started. Up to now, the microorganism such as bacteria, fungi, yeast, actinomycete, algae and virus all can be used to synthesize nanomaterials, including metal, semiconductor, quantum dots and alloy with different sizes and shapes.

Bacteria

In contrast to other kinds of microorganisms, bacteria were utilized to synthesize nanoparticles earlier. Due to the mild conditions, high yield and easy purification, bacteria become the most widely studied microorganism, with the title of "the factory of nanomaterials".

In 1996, Southam's group demonstrated that *B. subtilis* 168, when incubated with aqueous chlorauric acid solution, synthesized Au nanoparticles of size 5-25 nm. The obtained Au nanoparticles were octahedral. Transmission electron microscopy (TEM) confirmed that the nanoparticles distributed either in the periplasmic space or in the cell wall [8]. In 2009, Gurunathan's group synthesized gold nanocubes with the *B. licheniformis* after 48h of incubation. That performed at room temperature. The nanoparticles were polydisperse and the size ranged 10-100 nm [9]. There are also some reports about the synthesis of silver nanoparticles with the B. In 2008, Gurunathan's group investigated the synthesis of silver nanoparticles with *B. licheniformis*. The silver nanoparticles were well-dispersed and the size was about 50 nm [10]. In the same year, Sangiliyandi's group synthesized extracellular Ag nanoparticles with the supernatant of *B. licheniformis*. The size was proved to be approximately 40 nm [11]. In 2014, Banu's group utilized *B. thuringiensis* to synthesize silver nanoparticles with the size ranging from 43.52 to 142.97 nm. In addition, they confirmed that the Ag nanoparticles could be used as an effective larvicide against *Aedes aegypti* [12]. The B. can also be used to synthesize metal oxide nanoparticles. In 2011, Kim's group confirmed that cobalt oxide nanostructures could be synthesized with the Gram-positive bacteria, *B. subtilis*. As it reported, "rod-type cobalt oxide is prepared at room temperature through an electrostatic interaction between the functional surface of the bacteria and cobalt ions in aqueous solution". Moreover, porous hollow rods of Co_3O_4 were formed after a heat treatment at 300 degree. These transition metal oxides with special nanostructures have many practical applications. They can be used as catalysts, sensors, as well as batteries [13]. In the same year, Rahuman's group synthesized Titanium dioxide nanoparticles using the microbes *B. subtilis*. The nanoparticles were either spherical or oval in shape. The size ranged 66-77 nm [14].

Researchers also utilized *E. coli* to synthesize nanoparticles. In 2004, Iverson's group found that the intracellular cadmium sulfide nanocrystals could be synthesized when the *E. coli* incubated with sodium sulfide and cadmium chloride. The size ranged between 2-5 nm. The most significant was that they confirmed the synthetic yield was related to the cell cycle. In stationary phase the *E. coli* synthesized more nanocrystals than those in late logarithmic phase [15]. In 2007, Erkang Wang's group found the *E.*

coli DH5 α could synthesize gold nanoparticles on the surface. Most of the nanoparticles were spherical. More importantly, they discovered that the bio-nanocomposite could fix the hemoglobin which could be used to manufacture the membrane electrode. In this way, they achieved the direct electron metastasis of hemoglobin [16]. In 2010, Chang Ming Li's group harvested extracellular cadmium telluride with good crystallinity using the *E. coli*. The cadmium telluride is one kind of quantum dots and has tunable fluorescence emission. Further studies showed that the CdTe QDs was covered with protein molecules which formed the capping layer. That may improve the biocompatibility of the CdTe QDs. Furthermore; the biosynthesized CdTe has many applications such as bio-imaging and bio-labeling [17]. In 2012, Attard's group manufactured platinum nanoparticles with *E. coli* MC4100. There were two types of biosynthesized platinum nanoparticles depending on the metal loading. The platinum nanoparticles were typically 1.6-3.0 nm (1% loading) and 3.8-5.2 nm (20% loading) [18].

The aerobic *Actinobacter sp.* is a kind of Gram-negative bacteria. There are also some reports about the biosynthesis nanoparticles using these bacteria. In 2005, Sastry's group discovered the *A. sp.* could be applied to synthesize iron oxide nanoparticles. The reaction was conducted in the aqueous potassium ferricyanide /ferrocyanide mixture at room temperature. These nanoparticles were composed by the maghemite and magnetite with high protein content (65 wt %). The research showed that after 24 h, the size of iron oxide nanoparticles ranged 10-40 nm of spherical shape. However, the size of nanoparticles ranged 50-150 nm of cubic shape after 48 h. Under the stimulation of iron precursor, the *A. sp.* would secrete two kinds of protein with molecular weights of 120 and 70 kDa, which had the ability to hydrolyze the precursor and synthesize iron oxide nanoparticles. However, there was no formation of nanoparticles when they experimented with some other microorganisms (*B. subtilis*, *Micrococcus luteus*, *Aerobacter aerogenes* and *E. coli*) in the same way [19]. In 2008, they carried out further research and confirmed that the *A. sp.* would respond differently due to different precursors. They manufactured iron sulfide when reacted with the aqueous solution of ferrous sulfide-ferric chloride and maghemite when exposed with ferric chloride under aerobic condition [20]. In the same year, Prasad's group obtained silicon/silica nanocomposites when the *A. sp.* was exposed to K_2SiF_6 precursor. The reductases and oxidizing enzymes produced under the stimulation of the precursor might play a key role in the biosynthesis process of silicon/silica nanocomposites. The nanocomposites were nontoxic to human skin cells [21].

The M. bacteria have been discovered for almost 40 years, but few researchers were working in this field until the last decade [22]. *Magnetospirillum gryphiswaldense* is first to be discovered that could synthesize intracellular magnetic nanoparticles with the size ranges between 5-120 nm. The main components of magnetosome crystals are generally Fe_3O_4 and Fe_3S_4 . In 2003, Schuler's group found a nonmagnetic mutant of the magnetotactic bacterium, designated strain *M. gryphiswaldense* MSR-1. The mutant lost the ability to resemble magnetosome crystals because of some structures lacked. Further studies demonstrated that the synthesis of magnetosome was controlled by some genes which were lacked in the mutant [23]. In 2004, they did further research on the biochemical analysis of the magnetosome membrane, showing that the MM-associated proteins may have relevance for the formation of magnetite and magnetosome assembly [24]. In 2013, Mallapragada's group reported a typical review, with a systematical explanation of the mechanism and application of magnetosome biomineralization, making us a better understand of magnetic nanomaterials [25].

Recently, the methods of using the *Geobacter sulfurreducens* to synthesize metal nanoparticles have been reported. In 2013, Liu's group firstly reported the reduction of Pd(II) using the *G. sulfurreducens*. The Pd nanoparticles were inside the periplasm and capable of reducing Cr(VI). They confirmed that the nanoparticle number, size and Cr(VI)-reducing activity were affected by anthraquinone-2, 6-disulfonate and the ratio of cell dry weight: Pd [26]. After a few months, Logan's group utilized the *G.*

sulfurreducens to synthesize palladium nanoparticles which were predominantly outside the cell, making it easier to obtain nanoparticles without cell destruction [27].

In 2009, Shahverdi's group successfully synthesized silver nanoparticles with an average size of 3 nm using the supernatant of *Klebsiella pneumonia*. Moreover, they discovered that no nanoparticles were obtained when the process carried out in the dark. Subsequently, they studied how liquid mixing and visible-light irradiation affect the synthesis of silver nanoparticles. They also investigated the mechanism partially, considering the silver chloride was an important intermediate compound [28]. Next year, they firstly utilized the *K. pneumonia* to synthesize selenium nanoparticles. The size of nanoparticles obtained ranged between 100-550 nm. They confirmed that the selenium nanoparticles were chemically stable during the process of wet heat sterilization, making it easy to separate nanoparticles from the cell of bacteria [29].

The TiO₂ nanoparticles with a strong effect of UV shielding can be used in the field of cosmetics, functional fibers, plastics, coatings, paint, etc. In 2009, Prasad's group obtained TiO₂ nanoparticles using the *Lactobacillus sp.* which is a kind of Gram-positive bacterium. The process took place at room temperature. As for the mechanism, they proposed that "the synthesis of *n*-TiO₂ might have resulted due to pH-sensitive membrane bound oxidoreductases and carbon source dependent rH₂ in the culture solution". However, further studies are needed to recognize the specific mechanism [30].

In 2011, Bhargava's group firstly showed that different shape of silver nanoparticles could be achieved by controlling the growth kinetics of *Morganella psychrotolerans*, which were grown at four different temperatures. They discovered that primarily spherical silver nanoparticles with the size ranged between 2-5 nm were obtained at the optimum growth temperature of 20°C. When the temperature turned into 25°C and 15°C, "a mixture of triangular and hexagonal nanoplates along with spherical nanoparticles was obtained". However, the number of nanoplates increased significantly at 4°C, whereas only a small proportion of spherical nanoparticles were obtained. Furthermore, they performed electrochemistry experiments on the bacteria after their exposure to silver ions for the first time, providing an interesting potential mechanism of Ag ions reduction. Initially the silver ions were taken up by the bacteria cells, which underwent reduction in the role of proteins or other biomacromolecules and later released out of the cells (Figure 13.1). But how growth temperature affected the formation of anisotropic silver nanoparticles is not absolutely clear [31].

There are also many other bacteria that could be utilized to synthesize different nanoparticles. In 1993, Lundie's group discovered that *Clostridium thermoaceticum* synthesized Cadmium sulfide at the surface of cells when incubated with Cadmium chloride in the existence of cysteine which acted as a source of sulfide [32]. In 2000, Banfield's group found "abundant, micrometer-scale, spherical aggregates of 2- to 5-nanometer-diameter sphalerite (ZnS) particles formed within natural biofilms dominated by relatively aerotolerant sulfate-reducing bacteria of the family Desulfobacteriaceae" [33]. In 2006, Fredrickson's group discovered that extracellular UO₂ nanoparticles were formed by *Shewanella oneidensis* MR-1 depending on the c-type cytochromes [34]. Konishi's group utilized *S. algae* to synthesize intracellular gold nanoparticles of which the size ranged between 10-20 nm in the same year [35]. In the next year, they again obtained platinum nanoparticles using the *S. algae* at neutral pH and room temperature. The nanoparticles were about 5 nm and located in the periplasm [36]. A few months later, Mahmoud's group confirmed that extracellular gold nanoparticles with the size ranging between 15-30 nm could be synthesized by the *Pseudomonas aeruginosa* [37]. In 2008, Go's group first reported the biosynthesis method of gold nanowires using the extract of *Rhodospseudomonas capsulate*. Spherical gold nanoparticles with the size ranging between 10-20 nm were obtained, while gold nanowires with a network structure were formed when increased the concentration of Chlorauric acid [38]. In 2009, Bai' group demonstrated cadmium sulfide nanoparticles could be synthesized by *R. palustris* using the cadmium sulfate as a precursor. The size ranged between

7.76-8.26 nm. The cysteine desulphydrase which was in the cytoplasm acted as the sulfur source and the proteins secreted by the bacteria could stabilize the nanoparticles [39].

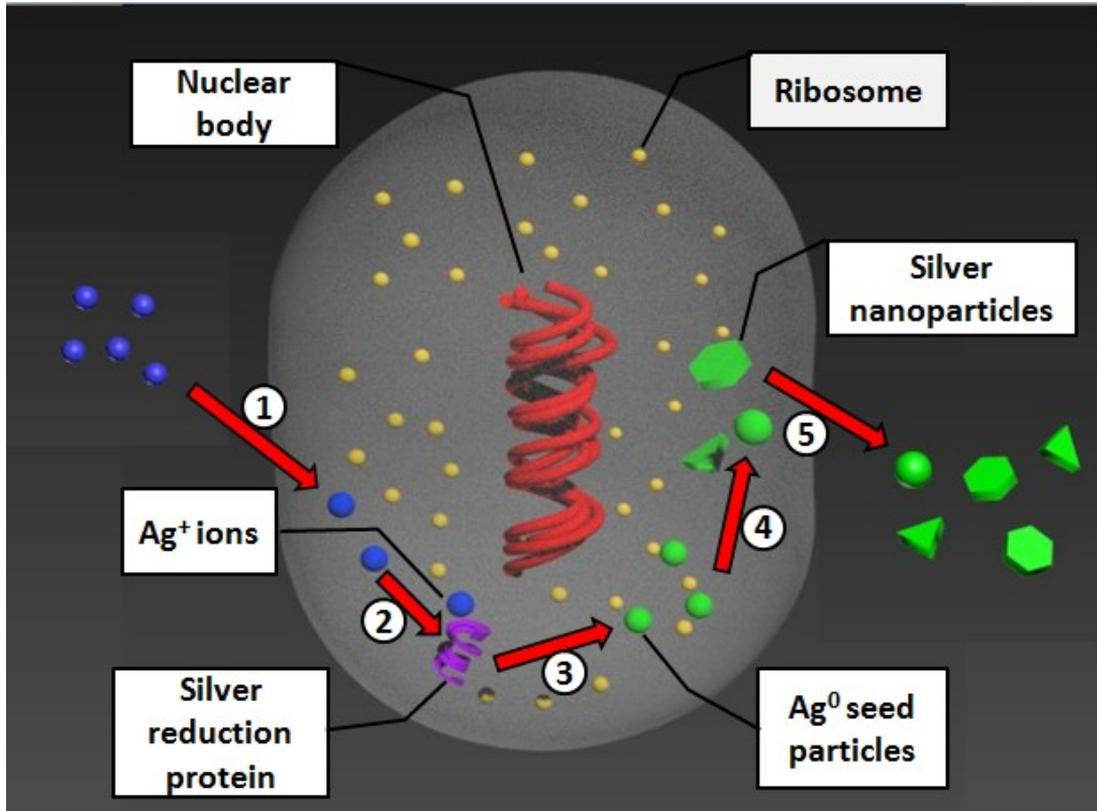


FIGURE 13.2

Potential mechanism for extracellular silver nanoparticles synthesis by *M. psychrotolerans*. (1) Ag⁺ ions were taken in by bacterial cell. (2) Silver reduction proteins bound to Ag⁺ ions. (3) Ag⁺ ions were reduced to Ag⁰ nuclei and seed nanoparticles. (4) The silver seed nanoparticles underwent growth and assembly leading to Ag nanoparticles in different shapes. (5) The nanoparticles were released out the bacterial cell

Fungi

Recently, fungi have been attracting more attention for the synthesis of different types of nanoparticles [40]. As the fungi could secrete plenty of enzymes and are easier to handle than other microorganisms, the biosynthesis of nanoparticles using fungi is potentially exciting. However, the genetic manipulation which can be a means of over expression of some specific enzymes is much more difficult than in prokaryotes.

Compared with other fungi, *Fusarium oxysporum* are relatively more often used for the biosynthesis of nanoparticles. In 2002, Khan's group discovered that *F. oxysporum* could synthesize gold nanoparticles extracellularly. The spherical or triangular gold nanoparticles of size 20-40 nm were well-dispersed and no significant reunion was found even after a month [41]. In the same year they firstly reported that

stable cadmium sulfide was synthesized under the action of the sulfate reductases secreted by *F. oxysporum* when exposed to CdSO_4 solution. The size of the well-dispersed CdS nanoparticles ranged between 5-20 nm. Similarly, ZnS, PbS and MoS_2 nanoparticles also could be synthesized by *F. oxysporum* when incubated with corresponding sulfate [42]. One year later, they again obtained extracellular silver nanoparticles of 5-15 nm using the aforementioned fungus. The nanoparticles were stabilized by proteins secreted by these fungi in solution [43]. About half a year later, Ahmad's group performed the biosynthesis of cruciform-shaped CaCO_3 crystals using *F. oxysporum* when simply exposed to aqueous calcium ions. They thought the proteins secreted by the fungi played a crucial role in the synthesis process [44]. In 2004, Sastry's group investigated the formation of zirconia nanoparticles using the fungus *F. oxysporum*, which was caused by the exposure of the fungus to aqueous solution of K_2ZrF_6 and subsequently protein-mediated hydrolysis of ZrF_6^{2-} anions at room temperature [45]. In 2005, Khan's group utilized the *F. oxysporum* to synthesize alloy nanoparticles and surprisingly Au-Ag alloy nanoparticles were obtained which was the first report on the fungus-mediated biosynthesis of Au-Ag alloy nanoparticles. The highly stable alloy nanoparticles in the size range 8-14 nm were formed when the fungi were exposed to equimolar concentrations of AgNO_3 and HAuCl_4 . *F. oxysporum* was demonstrated to secrete NADH-dependent enzymes and they confirmed that the nanoalloy composition was different in the control of the amount of NADH [46]. In 2006, Ahmad's group achieved that the substantial amorphous hydrated silica in the rice husk could be bioleached and biotransformed into quasi-spherical crystalline silica nanoparticles in the size of 2-6 nm. Further study demonstrated that the amorphous hydrated silica could be bioleached by the proteins secreted by the cells; however, amorphous hydrated silica could not be biotransformed into silica nanocrystallites by these proteins alone [47]. In another research, they firstly showed that ferroelectric barium titanate of 4-5 nm size could be synthesized at room temperature when aqueous solutions of potassium hexafluorotitanate and barium acetate reacted with *F. oxysporum*. These nanoparticles exhibited well-defined ferroelectric-paraelectric transition [48]. In fact, they also achieved the extracellular biosynthesis of magnetite using the *F. oxysporum* [49].

In 2012, Ahmad and Syed showed that extracellular platinum nanoparticles with the size ranged between 5-30 nm were obtained when the *F. oxysporum* incubated with H_2PtCl_6 . The nanoparticles were stabilized by proteins [50]. Last year, they investigated for the first time the biosynthesis of CdTe nanoparticles by *F. oxysporum*. The CdTe quantum dots were highly fluorescent and capped by proteins secreted by the fungus. They showed antibacterial activity against Gram negative bacteria and Gram positive bacteria [51]. Almost the same time, Bakhtiari's group reported the biosynthesis of spherical ZnS nanoparticles with the average size of 42 nm using the *F. oxysporum* [52]. Some other *F. sp.* such as *F. acuminatum* [53] and *F. semitectum* [54] could be used to synthesize extracellular silver nanoparticles.

In 2013, El-Sonbaty synthesized silver nanoparticles by the aqueous extract of *Agaricus bisporus*. Further study revealed that these nanoparticles were of spherical shape with the size ranged between 8-20 nm. Interestingly, they demonstrated "the silver nanoparticles exhibited a dose-dependent cytotoxic effect on MCF-7 breast cancer cells", which might be a promising alternative agent for cancer treatment [55]. Similarly, Ahmad's group showed the silver nanoparticles synthesized by *Humicola sp.* were non-toxic to both normal and cancer cells up to the concentrations of 50 $\mu\text{g}/\text{ml}$ [56]. In the same year, Sakthivel's group investigated the biosynthesis of silver nanoparticles using *Cylindrocladium floridanum*, showing that the silver nanoparticles obtained might be used as catalyst in the reduction of 4-nitrophenol to 4-aminophenol by sodium borohydride [57]. There are many other fungi that could be utilized to synthesize silver nanoparticles, for example, *Verticillium* [7], *Aspergillus flavus* [58], *A. fumigates* [59], *A. terreus* [60], *Cladosporium cladosporioides* [61], *Epicoccum nigrum* [62], *Penicillium citrinum* [63], *P. fellutanum* [64], *Trichoderma asperellum* [65] and *T. harzianum* [66], etc.

There are also reports about the biosynthesis of gold nanoparticles by fungi. In 2014, Castillo's group achieved the formation of gold nanoparticles using the fungus *Botrytis cinerea* for the first time. The nanoparticles were in different shapes such as spherical, pyramidal, hexagonal, triangular and decahedral of sizes ranging between 1-100 nm. They confirmed the molecules secreted by the fungus participated in the production of gold nanoparticles, but the specific mechanism remains to be elucidated [67]. Others such as *Verticillium* [68], *A. fumigates* [69], *A. flavus* [69] *Rhizopus oryzae* [70] and *C. floridanum* [71] have also been reported about the ability to biosynthesize gold nanoparticles. In 2011, Castro-Longoria's group found the fungus *Neurospora crassa* could synthesize not only silver or gold nanoparticles but also alloy-type Au/Ag nanoparticles. Different volume ratios of AgNO₃ and HAuCl₄ aqueous solutions resulted in different sizes of the Au-Ag bimetallic nanoparticles [72]. In 2013, Tarafdar and Raliya achieved the biosynthesis of iron nanoparticles using the fungus *A. oryzae* TFR9. The size of nanoparticles ranged between 10-24.6 nm [73]. A few months later, Ahmad and Khan reported for the first time the synthesis of cerium oxide nanoparticles when the fungus *H. sp.* incubated with the precursor cerium (III) nitrate hexahydrate. The average size of particles obtained was about 16 nm. The application of cerium oxide nanoparticles in biomedicine is still under research [74].

Yeast

The reports about the biosynthesis of metallic nanoparticles using the yeast are fewer than bacteria and fungi. Moreover, most of the reports are about the synthesis of quantum semiconductor nanocrystallites. Early as the 2002, Paknikar's group achieved the intracellular synthesis of PbS nanoparticles with the size ranged between 2-5 nm using the *Torulopsis sp.* for the first time [75]. In another research, they successfully synthesized intracellular cadmium sulfide nanoparticles with the size ranged between 1-1.5 nm by the *Schizosaccharomyces pombe* [76]. In 2003, they reported the extracellular synthesis of silver nanoparticles using the yeast strain MKY3. The nanoparticles obtained were in the size range of 2-5 nm [77].

As far as we know, the yeast *Sachharomyces cerevisiae* has been widely used for the biosynthesis of metallic nanoparticles in recent years. In 2009, Prasad's group investigated the biosynthesis of TiO₂ nanoparticles using the *S. cerevisiae*. The mean size of nano-sized TiO₂ was 12.57 ± 0.22 nm. They also proposed that the quinones and membrane bound oxidoreductases might affect the formation of TiO₂ nanoparticles [30]. In another two studies, they synthesized spherical Sb₂O₃ nanoparticles with the size of 2-10 nm [78] and spherical CdS nanoparticles with the average size of 3.57 ± 0.21 nm [79]. Furthermore, they reported the biosynthesis of PbS nanoparticles using the same yeast in 2012. The nano-sized PbS having the size of 0.667-6.95 nm were spherical in shape [80]. In 2009, Cui's group investigated the synthesis of CdSe nanoparticles. Excitingly, they confirmed that "CdSe QDs with different sizes and fluorescence wavelengths could purposely be obtained simply by controlling the incubation duration of the yeast cells with CdCl₂", which was quite different from the previous reports on the biosynthesis of PbS and CdS nanoparticles [81]. In 2010, Zhao's group demonstrated the biosynthesis of cadmium telluride quantum dots using *S. cerevisiae*. The resulting nanoparticles with size of 2-3.6 nm were capped with proteins, had good biocompatibility and might be used in the field of bio-imaging and bio-labeling [82]. In 2011, Mishra's group obtained silver and gold nanoparticles using the culture supernatant of *S. cerevisiae*. The size of silver and gold nanoparticles ranged between 5-20 nm and 20-100 nm respectively. Their further research confirmed that the optimum pH of the synthesis of silver and gold nanoparticles ranged between 8-10 and 4-6 respectively [83]. In 2014, Rose's group found the formation of ZnS quantum dot nanoparticles when the *S. cerevisiae* MTCC 2918 incubated

with zinc sulfate solution at room temperature. However, the nanoparticles of size range 30-40 nm were in low yield. The extraction of ZnS nanoparticles could be achieved by a freeze-thaw procedure [84].

Other yeast are capable of biosynthesizing metallic nanoparticles, for example, the yeast *Yarrowia lipolytica* NCIM 3589 was used to synthesize gold nanoparticles [85] and the *Rhodospiridium diobovatum* was used to synthesize lead sulfide nanoparticles [86].

Actinomycetes

Actinomycetes are widely distributed in nature, which has close relations with human production and life. It is the microorganisms that owns characteristics of both fungi and bacteria, has been famous for “radioactive fungus”. Compared with bacteria and fungi, actinomycetes were applied to nanometer biosynthesis research later. In 2003, Ahmad’s group first reported the actinomycetes also can be used to synthesis nanomaterials [87]. The gold nanoparticles were synthesized successfully with *Rhodococcus* sp. which is one kind of actinomycetes. They used alkalotolerant actinomycete to intracellular synthesis of gold nanoparticles with the dimensions of 5-15 nm. Electron microscope analysis showed that it has good monodispersity in both cell wall and cytoplasmic membrane. Particles are mainly concentrated on the cytoplasmic membrane. Furthermore, there is no toxic to the cells and it can grow well after biosynthesis of gold nanoparticles. Almost at the same time, with another actinomycete *Thermomonospora* sp, Ahmad’s group synthesized nanoparticles successfully [88]. That is the first report on the extracellular synthesis of gold nanoparticle with a prokaryotic microorganism. It is a kind of thermoactinomycetes, which can restore gold ions into spherical nanoparticles with good monodispersity and the average size is 8nm. FTIR analysis showed that the surface of the gold nanoparticles package is the amide (N) and (O) protein as a stable factor, which can promote the nanoparticles.

In 2012, Ahmad’s and his team member prepared gold nanoparticles in the intracellular of alga *Tetraselmis kochinensis*, which might apply in the area of drug delivery, biomedical applications and catalysis [89]. Different from the results from actinomycetes, they discovered that compared with cytoplasmic membrane, the obtained nanoparticles were more concentrated upon the cell wall. In 2012, Pawar’s group also studied the versatile actinobacteria *R. sp.* [90]. They prepared silver nanoparticles through reducing aqueous silver nitrate. The AgNPs were characterized and showed spherical particles with an average size of 10 nm. The SAED pattern confirmed that these nanoparticles are crystalline in nature. It is exciting in 2011 that Balagurunathan and his group reported a new actinomycete *Streptomyces viridogens* strain [HM10] for the biosynthesis of gold nanoparticles [91]. Out of 10 actinomycete strains were tested, while the strain [HM10] exhibited the best potency in intracellular synthesis of gold nanoparticles. Presence of spherical and rod shaped gold nanoparticles in mycelium of the strain HM10 was determined by transmission electron microscopy and X-ray diffraction analysis, whereas their average size was 18-20nm. In addition, “the strain HM10 showed enhanced growth at 1 and 10 mM concentration of HAuCl_4 . The gold nanoparticles synthesized by the strain Hm10 showed good antibacterial activity against *S.aureus* and *E.coli* in well-diffusion method. The potential actinomycete HM10 strain was phenotypically characterized and identified as *S. viridogens* (HM10). One year later, Prakasham’s group also synthesized nanoparticles using Marine Isolate *S. albidoflavus* [92]. They can be produced in both extracellular and intracellular. Besides, they found an interesting phenomenon that particles synthesized by this strain were reaction time dependent. The mean size of nanoparticles was 14.5nm. Almost at the same time, Kyusik’s group also produced gold nanoparticles with *S. hygrosopicus* cells [93]. Multidimensional AuNPs were generated

through different pH and reaction time. With cyclic voltammetry analysis and a minimal inhibitory concentration assay, they have demonstrated the electrochemical and antibacterial properties of AuNPs. In 2012, Anbarasu's group used a novel *S. sp.* BDUKAS10 which is an isolate of mangrove sediment and they found a particular procedure for the biosynthesis of bactericidal silver nanoparticles [94]. This research showed that *S. sp.* BDUKAS10 is beneficial for synthesis of AgNPs through extracellular method according to the sustainable and ecofriendly process. Subsequently, Zonooz and his group fabricated gold nanoparticles using H₂AuCl₄ by *S. sp.* ERI-3 cell-free supernatant [95], which were formed after 48 h of incubation at 28 degrees C and pH 7. They evaluated the effects of different reaction parameters including incubation temperature, reaction time, H₂AuCl₄ concentration and pH on gold nanoparticles production [96], which optimized the reaction medium parameters to enhance synthesis rate of the nanoparticles for the first time.

In 2013, more and more researchers were interested in biosynthesis of nanoparticles by actinomycetes. Bhaskara's group synthesized gold nanoparticles by *S. sp.* LK-3 (JF710608) [97], which were found within size range of 5-50 nm. Au-N-LK3 treatment in *Plasmodium berghei* ANKA (PbA) infected mice delayed the parasitemia rise (~ 6%) compared to PbA infection on 8 days post infection. The results obtained suggested that the Au-N-LK3 possess anti-malarial activity and could be considered as a potential source for anti-malarial drug development. In 2013, Se-Kwon Kim's group used a novel *Nocardiosis sp.* MBRC-1 for the synthesis of silver nanoparticles [98]. They were characterized by UV-visible, TEM, FE-SEM, EDX, FTIR, and XRD spectroscopy. Spherical nanoparticles were prepared with an average size of 45 ± 0.15 nm. The EDX analysis showed the presence of elemental silver signal in the synthesized nanoparticles, while XRD data exhibited those nanoparticles were crystalline in nature. The most important discovery is that the nanoparticles exhibited strong antimicrobial activity against bacteria and fungi and they claimed "Cytotoxicity of biosynthesized AgNPs against in vitro human cervical cancer cell line (HeLa) showed a dose-response activity IC₅₀ value was found to be 200 μ g/ml of AgNPs against HeLa cancer cells". Neelu Nawani's group studied the synthesis of gold nanoparticles from chloroauric acid using cell free supernatant of *S. sp.* NK 52 grown in nutrient broth [99]. This is the first report on the rapid biosynthesis of Au-NP using cell free supernatant of *S. sp.* and his evaluation for anti-lipid peroxidation.

Recently, El-Naggar studied four process variables (AgNO₃ concentration, incubation period, pH level and inoculum size) on the extracellular biosynthesis of silver nanoparticles by *S. viridochromogenes* [100]. The maximum biosynthesis of silver nanoparticles was achieved at a concentration of 0.5% (v/v) of 1 mM AgNO₃, incubation period of 96 h, initial pH of 9 and inoculum size of 2% (v/v). Compared to the cell-free supernatant, the biosynthesized AgNPs revealed superior antimicrobial activity against Gram-negative, Gram-positive bacterial strains and *Candida albicans*.

Algae

The study of biosynthesis nanoparticles using algae began comparatively late, which mainly focuses on the synthesis of nano gold and silver nanoparticles. In 2007, G. Singaravelu and his group could quickly mediate extracellular synthesis of 8-12nm gold nanoparticles with extraction of *Sargassum wightii* Greville [101], which is the first report in which a marine alga has been used to synthesize highly stable extracellular gold nanoparticles in a relatively short time period compared with that of other biological procedures. Jianping Xie's group produced single-crystalline gold nanoplates by treating an aqueous solution of chloroauric acid with the extract of the unicellular green alga *Chlorella vulgaris* at room temperature [102]. Similarly, Govindaraju produced silver nanoparticles with extracellular synthesis by brown seaweed [103]. In 2010, Rajasulochana's group reported the reaction condition of the alga *K.*

alvarezii with aqueous gold ions for gold nanoparticle synthesis within the biomass extracellularly [104]. Consequently, Brayner found that *Klebsormidium flaccidum* algal cells exhibiting the ability to form gold nanoparticles intracellularly in suspension were encapsulated within silica gels [105]. In 2011, Subhash Chandra Yadav exploited unicellular algae *Chlamydomonas reinhardtii* as a model system to elucidate the role of cellular proteins in SNPs biosynthesis [106]. Subsequently, Balasubramanian's group explored the biosynthesis of Au (0) from Au (III) using a new brown marine alga *T. conoides* using a combination of biosorption and subsequent bioreduction processes [107]. In 2012, Anantharaman reported the Biological synthesis of gold nanoparticles by brown alga, *Stoechospermum marginatum* biomasses through a green route was reported in this study [108]. Similarly, Ahmad's group studied the intracellular synthesis of gold nanoparticles of dimensions 5-35 nm using alga *T. kochinensis* [89]. Recently, Ahmad and his group used the brown marine algae *Sargassum muticum* aqueous to synthesis nanostructure silver particles for the first time [109].

Virus

Different from other microorganism, viruses are smaller than bacteria, no cell structure, which is a kind of microorganism only by proliferating in living cells. The reports for synthesis of nanoparticles by viruses are rarely. In 1998, Douglas's group found that cowpea chlorotic mottle virus can be used as a host for the synthesis of materials [110]. Subsequently, Mann and his group reported a new approach to the template-directed synthesis of inorganic-organic nanotubes using tobacco mosaic virus [111]. In 2002, Douglas proved that "assembled viral protein cages can be altered by design to provide organic scaffolding for inorganic nanomaterials synthesis constrained within the cage structure" [112]. They created a new cage with specific chemical functionality using the protein cage of the well-characterized cowpea chlorotic mottle virus. In a short time, Belcher fabricated a highly ordered composite material using a liquid crystal system from genetically engineered M13 bacteriophage and zinc sulfide nanocrystals [113]. The bacteriophage has a specific recognition moiety for ZnS crystal surfaces. In 2003, Chuanbin Mao's group found a new synthesis route to a semiconductor nanoscale heterostructure using M13 bacteriophage [114]. Recently in 2013, Yoon Sung Nam and his group reported the synthesis of a high-performance, flexible nanogenerator using anisotropic BaTiO₃ nanocrystals on an M13 viral template by the genetically programmed self-assembly of metal ion precursors [115]. Their study showed that "the biotemplating approach as a facile method to design and fabricate nano-scaled materials particularly suitable for flexible energy harvesting applications."

Conclusions

The synthesis of metallic nanoparticles using microorganisms including bacteria, fungi, yeast, actinomycetes, algae and viruses was introduced. Biosynthesis of nanoparticles is a promising, green, non-toxic and environmental friendly method. Most of the process can be performed at ambient temperature and pressure and also at neutral pH. After the synthesis of nanoparticles, microorganisms usually still have the ability to reproduce, which indicates that the nanoparticles synthesized by microorganisms show good biocompatibility. Based on these advantages, the synthesis of metallic nanoparticles by microorganisms is receiving increased attention.

The nanoparticles or nanostructures have numerous applications in many fields. Silver nanoparticles synthesized by *B. thuringiensis* could be used as an effective larvicide against *Aedes aegypti* [12], the

porous hollow rods of Co_3O_4 nanostructure synthesized by *B. subtilis* could be used as catalysts, sensors, as well as batteries [13], the CdTe quantum dots have many applications such as bio-imaging and bio-labeling [17]. Xiurong Yang and Hui Zhu successfully testified a hydrothermal assistant pyrolysis procedure to synthesize the derivative carbon with promising performance in capacitors [116]. The study introduced a facile strategy to discover carbonaceous materials and triggers interest in exploring fungi for material science applications. Besides, they might also be used in diverse areas such as cosmetics, coatings and packaging. As they have good biocompatibility, they will have broad application prospects in the biomedical field.

Many of the areas still require continued investigation. The focus of research in the near future should include: (1) The controlled synthesis and assembling of nanomaterials with a certain morphology and structure should be more professionally effective. (2) The detailed mechanism for the nanoparticles produced by microorganism remains to be elucidated. (3) More simple, feasible and environmentally friendly new synthetic routes to prepare alloy nanoparticles are under progress. (4) Some microorganism engineered nanoparticles might exhibit new and unexpected biological properties. Unfortunately, the current investigations are still concentrated on the process of synthesis, further studies on the practical applications of the obtained materials, especially in biomedical area, should be undertaken. Studies on the possible side effects and long-term toxicity of these rising materials, which are crucial to their real utilizations, must be carried out systematically, regularly and objectively.

TABLE 13.1

Microbial sources for synthesis of nanoparticles (In, Ex and NF are the abbreviations of “intracellular”, “extracellular” and “not found” respectively)

Microorganism	Type of nanoparticle	Morphology	Size range(nm)	Location	Reference
Bacteria					
<i>Bacillus subtilis</i>	Au	octahedral	5-25	In	[8]
	Co_3O_4	spherical	2-5	Ex	[13]
	TiO_2	spherical, oval	66-77	NF	[14]
<i>Bacillus licheniformis</i>	Au	cube	10-100	NF	[9]
	Ag	NF	40	Ex	[11]
<i>Escherichia coli</i>	CdS	NF	2-5	In	[15]
	CdTe	spherical	2-3	Ex	[17]
<i>Escherichia coli DH5α</i>	Au	spherical	25-33	In	[16]
<i>Escherichia coli MC4100</i>	Pt	spherical	1.6-3.0/3.8-5.2	NF	[18]
<i>Actinobacter sp.</i>	Magnetite	cube	50-150	Ex	[19]
	Si/SiO ₂	spherical	NF	Ex	[21]
<i>Magnetospirillum gryphiswaldense</i>	$\text{Fe}_3\text{O}_4/\text{Fe}_3\text{S}_4$	cube/octahedral	45	In	[23]

<i>Geobacter sulfurreducens</i>	Pd	NF	11-17	Ex	[27]
<i>Klebsiella pneumonia</i>	Ag	NF	1-6	Ex	[28]
	Se	spherical	100-550	In	[29]
<i>Lactobacillus sp.</i>	TiO ₂	spherical	24.31-24.95	Ex	[30]
<i>Morganella psychrotolerans</i>	Ag	spherical	2-5	Ex	[31]
<i>Clostridium thermoaceticum</i>	CdS	NF	NF	Ex	[32]
<i>Desulfobacteriaceae</i>	ZnS	spherical	2-5	Periplasm	[33]
<i>Shewanella oneidensis MR-1</i>	UO ₂	NF	1-5	Ex	[34]
<i>Shewanella algae</i>	Au	NF	10-20	In	[35]
	Pt	NF	5	Periplasm	[36]
<i>Pseudomonas aeruginosa</i>	Au	NF	15-30	Ex	[37]
<i>Rhodopseudomonas capsulate</i>	Au	spherical	10-20	Ex	[38]
<i>Rhodopseudomonas palustris</i>	CdS	spherical	7.76-8.26	In	[39]

Microorganism	Type of nanoparticle	Morphology	Size range(nm)	Location	Reference
Fungi					
<i>Fusarium oxysporum</i>	Au	spherical/triangular	20-40	Ex	[41]
	CdS	hexagonal	5-20	Ex	[42]
	Ag	spherical/triangular	5-15	Ex	[43]
	CaCO ₃	cruciform	NF	Ex	[44]
	ZrO ₂	quasi-spherical	3-11	Ex	[45]
	Au-Ag	spherical	8-14	Ex	[46]
	SiO ₂	quasi-spherical	2-6	Ex	[47]
	BaTiO ₃	quasi-spherical	4-5	Ex	[48]
	Magnetite	quasi-spherical	20-50	Ex	[49]
	Pt	spherical	5-30	Ex	[50]
	CdTe	spherical	15-20	Ex	[51]
ZnS	spherical	42	Ex	[52]	

<i>Fusarium acuminatum</i>	Ag	spherical	5-40	Ex	[53]
<i>Fusarium semitectum</i>	Ag	spherical	10-60	Ex	[54]
<i>Agaricus bisporus</i>	Ag	spherical	8-20	Ex	[55]
<i>Humicola sp.</i>	Ag	spherical	5-25	Ex	[56]
	CeO ₂	spherical	16	Ex	[74]
<i>Cylindrocladium floridanum</i>	Ag	spherical	25	Ex	[57]
	Au	spherical	19.05	Ex	[71]
<i>Verticillium</i>	Ag	spherical	13-37	In	[7]
	Au	spherical/triangular hexagonal	12-28	In	[68]
<i>Asperigillus flavus</i>	Ag	spherical	7.31-10.53	Ex	[58]
	Au	spherical/triangular hexagonal	17.76/22.61	Ex/In	[69]
<i>Aspergillus fumigatus</i>	Ag	spherical/triangular	5-25	Ex	[59]
	Au	spherical/triangular hexagonal	17.76/26.16	Ex/In	[69]
<i>Aspergillus terreus</i>	Ag	spherical	1-20	Ex	[60]
<i>Cladosporium cladosporioides</i>	Ag	spherical	10-100	Ex	[61]
<i>Epicoccum nigrum</i>	Ag	spherical	1-22	Ex	[62]
<i>Penicillium citrinum</i>	Ag	spherical	109	Ex	[63]
<i>Penicillium fellutanum</i>	Ag	spherical	5-25	Ex	[64]
<i>Trichoderma asperellum</i>	Ag	spherical	13-18	Ex	[65]

Microorganism	Type of nanoparticle	Morphology	Size range(nm)	Location	Reference
Fungi					
<i>Trichoderma harzianum</i>	Ag	spherical	4.66	Ex	[66]
<i>Botrytis cinerea</i>	Au	spherical/pyramidal/hexagonal/triangular decahedral	1-100	Ex	[67]
<i>Rhizopus oryzae</i>	Au	spherical	10	Ex	[70]
<i>Neurospora crassa</i>	Au/Ag/Au-Ag	spherical	11/32	In	[72]

<i>Aspergillus oryzae</i> TFR9	Fe	spherical	10-24.6	Ex	[73]
Yeast					
<i>Torulopsis sp.</i>	PbS	spherical	2-5	Ex	[75]
<i>Schizosaccharomyces pombe</i>	CdS	hexagonal	1-1.5	Ex	[76]
Yeast strain MKY3	Ag	spherical	2-5	Ex	[77]
<i>Sachharomyces cerevisiae.</i>	TiO ₂	spherical	12.35-12.79	Ex	[30]
	Sb ₂ O ₃	spherical	2-10	In	[78]
	CdS	spherical	3.36-3.78	Ex	[79]
	PbS	spherical	0.667-6.95	NF	[80]
	CdSe	NF	2.69-6.34	In	[81]
	CdTe	cubic	2-3.6	Ex	[82]
	Ag	spherical	5-20	Ex	[83]
	Au	spherical	2-100	Ex	[83]
	ZnS	sphalerite	30-40	In	[84]
<i>Yarrowia lipolytica</i>	Au	hexagonal/triangular	15	In	[85]
<i>Rhodospiridium diobovatum</i>	PbS	cubic	2-5	In	[86]
Actinomycetes					
<i>Rhodococcus sp.</i>	Au	spherical	5–15	In	[87]
<i>Rhodococcus sp.</i>	Ag	spherical	10	In	[90]
<i>Thermomonospora sp</i>	Au	spherical	8	Ex	[88]
<i>Streptomyces viridogens</i>	Au	spherical	18-20	In	[91]
<i>Streptomyces albidoflavus</i>	Ag	spherical	14.5	Ex	[92]
<i>Streptomyces hygrosopicus</i>	Au	spherical	10–20	Ex	[93]

Microorganism	Type of nanoparticle	Morphology	Size range(nm)	Location	Reference
Actinomycetes					
<i>Streptomyces sp. BDUKAS10</i>	Ag	spherical	21–48	Ex	[94]
<i>Streptomyces sp. ERI-3</i>	Au	spherical	10–30	Ex	[96-96]

<i>Streptomyces sp LK-3</i>	Au	NF	5-50	NF	[97]
<i>Nocardiopsis sp. MBRC-1</i>	Ag	spherical	45±0.15	Ex	[98]
<i>Streptomyces sp NK 52</i>	Au	rods/hexagons/triangles/spheres	10-100	Ex	[99]
<i>Streptomyces viridochromogenes</i>	Ag	spherical	2.15-7.27	Ex	[100]
Alga					
<i>Tetraselmis kochinensis</i>	Au	spherical	5–35	In	[89]
<i>Sargassum</i>	Au	NF	8-12	Ex	[101]
<i>Chlorella</i>	Au	NF	1-1000	Ex	[102]
<i>Brown seaweed</i>	Ag	NF	NF	Ex	[103]
<i>Kappaphycus alvarezii</i>	Au	spherical	NF	Ex	[104]
<i>Klebsormidium flaccidum</i>	Au	NF	NF	In	[105]
<i>Chlamydomonas reinhardtii</i>	Ag	spherical	5-15	Ex	[106]
<i>Turbinaria conoides</i>	Au	spherical	5-15	Ex	[107]
<i>Stoechospermum marginatum</i>	Au	spherical/hexagonal/ triangle	18.7-93.7	NF	[108]
<i>Sargassum muticum</i>	Ag	spherical	5-15	NF	[109]
Virus					
<i>Tobacco Mosaic Virus</i>	SiO ₂ /CdS/PbS Fe ₂ O ₃	NF	5	NF	[111]
<i>M13 bacteriophage</i>	ZnS, CdS	hexagonal	10-20	NF	[113-114]
<i>E3-M13</i>	BaTiO ₃	filamentous	50-70	NF	[115]

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