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Review Article

Marine microbes: Invisible nanofactories

Syed Baker^a, B.P. Harini^b, D. Rakshith^a, S. Satish^{a,*}^aHerbal Drug Technological Laboratory, Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore 570 006, Karnataka, India^bDepartment of Zoology, Bangalore University, Jnanabharathi Campus, Bangalore 560 056, Karnataka, India

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ABSTRACT

Biological synthesis of nanoparticles has emerged as rapidly developing research area in nanotechnology across the globe with various biological entities being employed in production of nanoparticles constantly forming an impute alternative for conventional methods. Simple prokaryotes to complex eukaryotic organisms including higher plants are used for the fabrication of nanoparticles. One area of untapped potential is marine microorganism as nanofactories to fabricate nanoparticles. Marine microorganisms are known to interact with metal ions as marine ecosystems are constantly exposed to high metal salt concentration. These microorganisms may reduce the metallic ions rapidly for the formation of nanoparticles of desired shape and controlled size. The present review unearths marine microbial flora in synthesis of nanoparticles.

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1. Introduction

Nanotechnology can be defined as the design, synthesis, and application of materials and devices whose size and shape have been engineered at the nanoscale.¹ It exploits unique chemical, physical, electrical, and mechanical properties that emerge when matter is structured at the nanoscale. One of the most important aspects in nanotechnology relies on the synthesis of nanoparticles with well-defined sizes, shapes and controlled monodispersity. One of the major challenges of current nanotechnology is to develop reliable and non-toxic experimental protocols for the synthesis of nanoparticles with regards to non-toxic, clean and eco-friendly.² Biotechnological route has emerged as a safe and alternative process in synthesis of nanoparticles by employing ambient biological resources. Perusal of studies reported by far express biological synthesis of nanoparticles from simple prokaryotic organism

to multi cellular eukaryotes such as fungi and plants.^{3–6} The adaptation to heavy metal rich environments is resulting in microorganisms which express activities such as biosorption, bioprecipitation, extracellular sequestration, transport mechanisms, and chelation. Such resistance mechanism forms the basis for the use of microorganisms in production of nanoparticles. The strongly interdisciplinary field of microbiology and nanotechnology has upsurge nanoparticles synthesis spanning from the microorganism which holds much promise for applications of nanoparticles related to medical and pharmaceutical sciences.⁷ Microorganism isolated from array of habitats have expressed immense potential in production of nanoparticles one such habitat is marine. Marine microorganisms are known to thrive in unique niches such as tolerate high salt concentration, extreme atmospheric pressure etc. These microbes are known to have been explored with interest as source of novel bioactive factories

* Corresponding author. Tel.: +91 821 2419734 (O), +91 9448323129 (mobile).

E-mail address: satish.micro@gmail.com (S. Satish).

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synthesizing various functional metabolites displaying unique properties. However, these marine microbes are not sufficiently explored with regards to synthesis of nanoparticles few reports cited expressed the burgeoning interest among the researchers in exploiting the mechanisms of marine microbes for nanoparticle synthesis. As marine resource is one of the richest sources in the nature, marine microorganisms employed in production of nanoparticles are in infancy stage. Therefore, a possibility of exploring marine microbes as nanofactories forms a rational and reliable route in production of nanoparticles compared to the most popular conventional methods which are bound with limitations such as expensive, use of toxic elements in production protocols resulting limited applications in pharmaceutical and health sector. The present review envisions the role of marine microbes as emerging resource in synthesis of nanoparticles. The study also display so far reported marine microbial diversity in synthesis of nanoparticles, further research in this area will be promising enough to engulf the limitation of conventional methods forming a new avenue for rapid synthesis of nanoparticles with technical dimension.

2. Nanoparticles

Nanoparticles are particles with at least one dimension at nanoscale. Nanoparticles exist widely in the natural world as product of natural phenomena such as photochemical volcanic activity, ocean spray, forest-fire smoke, clouds and clay combustion and food cooking, and more recently from vehicle exhausts.³ Owing to their unique properties nanoparticles are known to have wide range of applications the potential of

nanoparticles is infinite with novel new applications constantly being explored.⁴

2.1. Synthesis of nanoparticles

Nanoparticles are synthesis by array of conventional methods which are divided into top down and bottom up processes (Fig. 1). In top down process the synthesis of nanoparticles from the bulk material is carried out by various lithographic techniques. In bottom up process is based on miniaturization at molecular level forming the nuclei and their growth into nanoparticles. These conventional methods are very popular and widely employed in synthesis of nanoparticles but are bounded with their own limitations such as expensive, use of high energy and use of hazardous toxic chemicals. Hence there is a burgeoning interest in eco-friendly process of nanoparticles production with precise control of size and desired shape.^{5–8}

2.2. Biosynthesis of nanoparticles

Biosynthesis of nanoparticles is a type of bottom up process in which biological entities are employed in synthesis of nanoparticles with large number of plants and microorganism reporting in facile synthesis of nanoparticles. Use of plants has been reported to produce nanoparticles of variable size and shape.⁹ But harvesting of endangered plant species can pose a risk and imbalance in the plant diversity hence research on microorganisms as ideal source in synthesis of nanoparticles has rapidly expanded with microorganism being isolated from various habitats and challenged with metal salts toward the unearthing nanoparticles production and this route has gained success with large species reporting

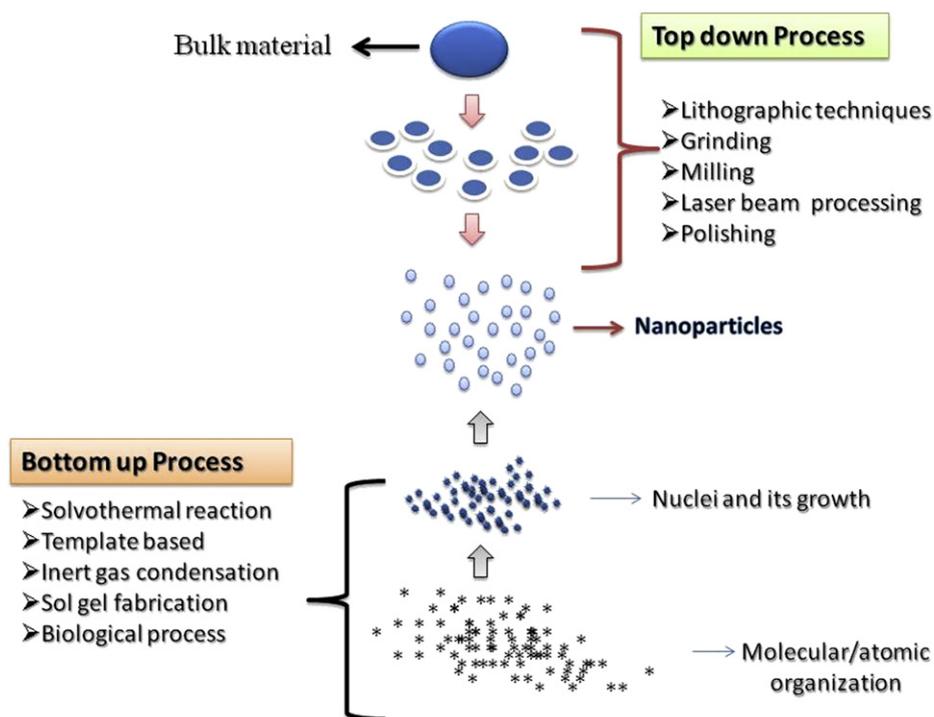


Fig. 1 – Synthesis of nanoparticles.

in production of nanoparticles with control size and desired shape (Table 1).

2.3. Microbial synthesis of nanoparticles

The role of microbes in synthesis of nanoparticles was first reported in 1984 by employing *Pseudomonas stutzeri* AG259, originally isolated from silver mine.¹⁰ Since then research on microbial synthesis of nanoparticles has expanded rapidly with one or the other reports confirming the production of nanoparticles by microorganism. The biological synthesis of nanoparticles originated by the experiment conducted by Mullen et al 1989 on biosorption of metals bacteria. The synthesized molecules were not identified as nanoparticles but as aggregates.¹¹ Microbes produce inorganic materials either intra or extracellular often in nanoscale dimensions with exquisite morphology. Microbial interactions between metals and microbes have been exploited for various biological applications in the fields of bioremediation, biomineralization,

bioleaching, and biocorrosion. The mechanism of microorganism tolerating metal ions has led microbial system as emerging source compared to other biological entities as facile route in nanoparticle production.^{12–14}

3. Marine microorganism as facile entity in synthesis of nanoparticles

Microorganisms forms huge diversity conquering extremely hostile environments which are being bioprospected as nature wealth for wide range of application one such burgeoning area is microbes propounded as source of nanofactories with array of microorganism being rapidly reported in synthesis of nanoparticles [Table 1] Microbial habitats forms a vital role, microbes characterized by extreme environmental conditions such as extreme pH, sparse nutrients, high metal content, intense salt load etc., are known to have unique mechanism for their existence. Marine habitat is one such resource bears a rich microbial flora with marine microorganisms these microbes are reported to have adapted toward unique mechanisms such as high salt concentration and can evade toxicity of different metal ions. Metal rich effluent is due to chemical reactions between marine water and mineral salts results in extreme environment.¹⁵ However, marine microbes acclimatize to such extreme condition for its survival. Exploiting such microbial resource for synthesis of nanoparticles will be promising enough as a facile bio-process. But reports of these microbes in synthesis nanoparticles are scanty with few reports representing the marine microbes in nanoparticles production. Hence the present review envisions and reports the marine microorganism in nanoparticle production by conferring the literature pursued so far.^{2,16}

4. Mechanism

The biogenic entities are found to secrete large amount of proteins which are found to be responsible for metal ion reduction and morphology control.¹⁷ In different microorganisms, various enzymes are believed to take part in the bioreduction process involving the transport of electrons from certain electron donors to metal electron acceptors. Some studies of non-enzymatic reduction mechanism suggested that some organic functional groups of microbial cell walls could be responsible for the bioreduction process.¹⁸ All the above mechanisms could result in the intracellular or extracellular complexation and the deposition of metal nanoparticles. Biogenic nanoparticles are toward a greener approach and environment friendly with no toxic hazardous chemical employed in synthesis protocol with synthesis process taking place at ambient temperature and pressure conditions.^{19–21}

Mean while marine microorganisms are reported to reduce the metallic ions and convert them into phosphates, sulfides, carbonates, and/or intracellularly sequester them with low molecular weight such as cysteine rich proteins glutathione or phytochelatins which are induced upon exposure to metals in biological system.^{22–26} The metal peptide interaction is another incentive to use the biosynthetic route for

Table 1 – Represent some of the most important microorganism in synthesis of nanoparticles.

Microorganism	Type of nanoparticles	Size
Bacteria		
<i>Aquaspirillum magnetotactic</i>	Fe ₃ O ₄	40–50 nm
<i>Bacillus subtilis</i>	Au	10 nm
<i>Corynebacterium</i> sp SH09	Ag	10–15 nm
<i>Desulfovibrio desulfuricans</i>	Pd	50 nm
<i>Escherichia coli</i>	Au	30–40 nm
<i>Lactobacillus</i> sp	Au, Ag, Au–Ag	20–50 nm
<i>Magnetotactic bacterium</i>	FeS ₂	7.5 nm
<i>Pseudomonas aeruginosa</i>	Se, Ag	–
<i>Pseudomonas stutzeri</i> AG259	Ag	<200 nm
<i>Rhodobacter capsulatus</i>	Au	–
Fungi		
<i>Aspergillus flavus</i>	Ag	8–9 nm
<i>Aspergillus fumigatus</i>	Ag	5–25 nm
<i>Aspergillus niger</i>	Ag	20 nm
<i>Colletotrichum</i> sp.	Au	20–40 nm
<i>Coriolus versicolor</i>	Ag	25–75 nm
<i>Fusarium oxysporum</i>	CdSe	9–15 nm
<i>Fusarium acuminatum</i>	Ag	5–40 nm
<i>Fusarium oxysporum</i>	Bi ₂ O ₃	5–8 nm
<i>Fusarium oxysporum</i> f. <i>sp.lycopersici</i>	Pt	10–50 nm
<i>Fusarium semitectum</i>	Ag	10–60 nm
<i>Fusarium solani</i>	Ag	16–20 nm
Actinomycetes		
<i>Rhodococcus</i> sp	Au	5–15 nm
<i>Thermonospora</i> sp	Au	8 nm
Yeasts		
<i>P. jadinii</i>	Au	100 nm
<i>Saccharomyces cerevisiae</i>	Sb ₂ O ₃	3–10 nm
<i>Schizosaccharomyces pombe</i>	CdS	1–1.5 nm
<i>Yarrowia lipolytica</i>	Au	15 nm
Yeast MKY3	Ag	2–5 nm
<i>Candida glabrata</i>	PbS	<1 nm
Algae		
<i>Sargassum</i> algae	SiO ₂	50–100 nm
<i>Shewanella</i> algae	Au	10–20 nm

nanoparticle synthesis as capping of metal nanoparticles by peptides such as phytochelatins prevents aggregation into bulk crystals, thus yielding stable nanoparticles.²⁷

5. Marine microorganism reported in synthesis of nanoparticles

The variable biodiversity in the marine environment with that of the terrestrial environment influence researchers to exploit marine flora in array of applications, the interference between marine microbial systems and nanotechnology has opened a new avenue by employing marine microorganism in synthesis of nanoparticles. Based on the literature pursued it is reported that when two isolates of marine actinomycetes i.e., *Streptomyces parvulus* SSNP11 and *Streptomyces albidoflavus* CNP10 challenged with silver nitrate and incubate at 30 °C. The bio-reduction of the silver ions was associated with metabolic processes utilizing nitrate by reducing nitrate to nitrite and ammonium. The produced silver nanoparticles exhibited maximum absorbance at 400–410 nm in UV–Vis spectroscopy. The reaction products were analyzed using transmission electron microscopy, X-ray diffraction (XRD) and Fourier transform infrared spectroscopy. The study also reported that the production of silver nanoparticles was both intra and extracellular. The report also suggested that exposure to varying temperature, pH and substrate concentration influences, directly or indirectly, the rate of nanoparticles fabrication.²⁸

Similarly six fungal strains were isolated from marine mangrove sediment from Parangipettai. After culturing at 27 °C for 5 days the biomass was separated and washed with Milli Q water, 50 ml of cell filtrate was treated with 1 mM silver nitrate solution and incubated at 25 °C in dark. The nanoparticles production was expressed with an absorption peak at 420 nm in UV–Vis spectrum corresponding to the Plasmon resonance of silver nanoparticles thus confirming their presence. The Fourier transform infrared spectroscopy confirmed the presence of protein as stabilizing agent surrounding the silver nanoparticles.²⁹

In another report the bacterial *Pseudomonas* sp isolated from marine sample was cultured and treated with silver nitrate for synthesis of silver nanoparticles. Silver nanoparticles were obtained intracellularly which was characterized by UV-Spectrophotometer, X-Ray diffraction, and Scanning electron microscopy revealed the silver nano particles displayed poly dispersed with different sizes are ranging from 20 to 100 nm in size. XRD analysis showed that these nanoparticles exhibit a face-centered cubic crystal structure.³⁰

Similarly marine microalgae was collected from Central Marine Fisheries Research Institute and cultured in the laboratory and challenged with silver nitrate resulted in fabrication of silver nanoparticles by normal and microwave irradiation technique and the synthesized nanoparticles were evaluated for antimicrobial activity against human pathogens. The production of silver nanoparticle was confirmed by UV–Vis spectroscopy at 420 nm by the presence of Plasmon peak. Further confirmation was done by scanning electron microscope (SEM). These results not only provide a base for further research but still useful for drug development in the present and future.³¹

Yet another report performed by employing marine yeast *Candida* sp. VITDKGB isolated from Nicobar Islands, India. Production of silver nanoparticles was confirmed by the absorption peak at 430 nm in UV–Vis spectroscopy due to the surface Plasmon resonance of silver nanoparticles. Nanoparticles synthesized were characterized by atomic force microscopy, Fourier transform infrared spectroscopy and X-ray diffraction. The nanoparticles were evaluated for antimicrobial activity against multi drug resistant microorganism.³²

Similarly extracellular biosynthesis of silver nanoparticles was reported by employing marine cyanobacterium, *Oscillatoria williei* NTDM01 which reduces silver ions and stabilizes the silver nanoparticles by a secreted protein. The silver nitrate solution incubated with washed marine cyanobacteria resulted in formation of silver nanoparticles. The characteristics of the protein shell at 265 nm were observed in Ultra violet spectrum for the silver nanoparticles in solution. While FTIR analysis confirmed the presence of a protein shell which are responsible for the nanoparticles biosynthesis. Scanning electron microscopy revealed that the formation of agglomerated silver nanoparticles due to the capping agent in the range of 100–200 nm.³³

Apart from gold and silver nanoparticles being reported from marine microorganisms, lead nanoparticles was also reported using marine by marine yeast, *Rhodospiridium diobovatum*. The biosynthesis of lead nanoparticles was characterized by UV–Vis absorption spectroscopy, X-ray diffraction and energy dispersive atomic spectroscopy (EDAX). UV–Vis absorption scan revealed a peak at 320 nm. XRD confirmed the presence of nanoparticles of cubic structure and transmission electron microscopy revealed the nanoparticle formed were in the range of 2–5 nm.³⁴

With these literature reported so far unearths the new applications of marine microbial flora toward greener fabrication of nanoparticles. The present review is first of its kind conferring the reports of marine microbes in synthesis of nanoparticles. Further extensive research can be valuable with promising strains isolated from various niches of marine environment toward the synthesis of nanoparticles in future decades.

6. Protocols for microbial route synthesis of nanoparticles

Synthesis of nanoparticles protocol by microorganisms is broadly grouped into intracellular synthesis method and extracellular method (Fig. 2). In intracellular synthesis protocol the microbial cell or cell filtrate is employed and challenged with optimized metal salt concentration and incubated for synthesis of nanoparticles where as in extracellular synthesis protocol the supernatant obtained after harvesting the microbial cell is employed in the synthesis were in supernatant is challenged with metal salt concentration and incubated for production of nanoparticles. In both the protocols mentioned above physiological parameters such as pH, Temperature, Concentration of metal salts, Incubation type such as static or in shaker, Incubation period all play immense important role and influence synthesis of nanoparticles with precise shape and controlled size. Synthesis of nanoparticles are initially confirm by the UV–Visible spectral peak later the physiochemical

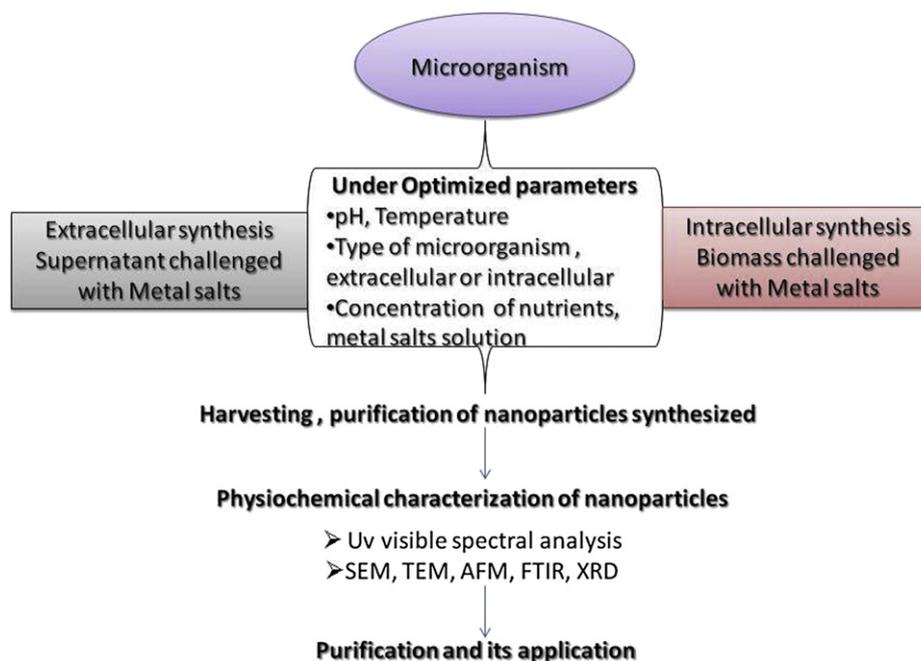


Fig. 2 – Protocol for biosynthesis of nanoparticles.

characteristics is carried out by various analytical microscopic techniques such as FTIR, XRD, SEM, TEM, AFM etc.^{16,35–47}

7. Conclusion

The recent development and implementation of new technologies has led to new era, the nano-revolution which unfolds role of biological synthesis of nanoparticles which seem to have drawn quite an unequivocal attention with a view of reformulating the green chemistry principle to develop eco-friendly production for nanoparticles which can be an alternative for most popular conventional methods. Among the biological employed microbes are being rapidly exploited from various niches for nanoparticle synthesis, the present study envisions toward exploiting marine microbial flora as emerging nanofactories. Further research in this area can open a new vista toward cellular, biochemical and molecular mechanisms that mediate the synthesis of biological nanoparticles.

Conflicts of interest

All authors have none to declare.

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