

Review Article

MICROALGAE MEDIATED SILVER NANOPARTICLES (AG-NPS) SYNTHESIS AND THEIR BIOLOGICAL ACTIVITIES

Rushikesh Dhavale, Supriya Jadhav and Sibi G*

Department of Biotechnology, Indian Academy Degree College-Autonomous, Bengaluru, India,

*Head of the Department, Department of Biotechnology, Indian Academy Degree College-Autonomous, Bengaluru, India
Email: gsibii@gmail.com

Received: 15.11.2019

Revised: 20.12.2019

Accepted: 25.01.2020

Abstract

Silver ions and silver based compounds are known bactericides. Silver nanoparticles (Ag-NPs) show efficient antibacterial activity due to the large surface area that comes in contact with the microbial cells and therefore, has a higher percentage of interaction than larger particles of the same parent material. With nanoparticles integrated in consumers' health and industrial products, it is necessary to develop techniques that implement a bio-synthesis of nanoparticles. Microalgae based nanoparticles have received attention due to their outstanding properties such as biocompatibility, biodegradability and low cost. In this study, microalgae as model biological systems for their ability to form Ag-NPs were reviewed. Further, biological activities of microalgae synthesized Ag-NPs as reported by earlier studies were evaluated.

Keywords: Microalgae, silver nanoparticles (Ag-NPs), antimicrobial, anticancer.

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DOI: <http://dx.doi.org/10.31838/jcr.07.02.04>

INTRODUCTION

The convergence confluence between nanotechnology and biology has developed a new field of nano biotechnology that reveals the use of biological items including algae and plants in a number of biochemical and biophysical processes [1]. The ability of a biological material to reduce inorganic metallic ions into metal-NPs using its internal biochemical processes has led to a comparatively new and uninvestigated area of research. These bio-nanofactories are able to significantly reduce environmental pollution [2]. Physicochemical synthesis of NPs is often cumbersome and costly with the release of harmful by-products posing a high risk to living systems [3, 4]. Since noble metal nanoparticles, such as gold, silver and platinum are widely applied to human contact areas, there is a growing need to develop environmentally friendly processes of nanoparticle synthesis that do not use toxic chemicals. Green formation of metal nanoparticles by naturally biodegradable components including polysaccharides, biopolymers, vitamins, plant extracts and microorganisms represent sustainable resources in biosynthesis of metal nanoparticles. Biological synthesis of nanoparticles using microbes, enzymes, plants, and algae has been proposed as an alternative to chemical and physical modes of synthesis. Microorganisms, both unicellular and multicellular, are known to produce inorganic materials, often of nanoscale dimensions, either intracellularly or extracellularly.

Among the lower organisms, microalgae have a tremendous role in bioremediation of toxic and precious metals and their bioconversion to different nontoxic forms [5]. Algae are known to hyper accumulate heavy metal ions and possess an exceptional capability to remodel them into more malleable forms [6, 7]. Some of the pragmatic properties of the algae that make them as remarkable 'nanobiofactories' are;

- faster doubling time [8]
- easily scalable and well developed systems [8, 9]
- cells can be readily disrupted [10]
- easily harvested [11]
- low cost large-scale synthesis [12] and

- nucleation and crystal growth are accelerated due to the presence of negative charge on the surface of the cell [13].
- Because of these alluring attributes, algae have been foreseen as model organisms for fabricating bio-nanomaterials. In general, algae-mediated synthesis of nanomaterials involves preparation of (i) algal extract, (ii) metal precursor solution, and (iii) incubation of algal extract with metal precursor solution [14].

Algae show more advantageous outcomes over other biological processes because it is more reasonable for large scale bio-production of metal-NPs. Initially, the nanoparticle synthesis was reported to be intracellular [15] but later algae were exploited for an extracellular mode of synthesis [7, 16, 17]. More than a hundred different micro and macro algae have been reported that exhibit the ability to tailor nanoparticles both intracellularly [18] and extracellularly [19].

Nanoparticles present a higher surface to volume ratio with their decreasing size. Among the various noble metallic NPs known so far silver NPs (Ag-NPs) have gained the most attention, exhibiting the highest level of commercialization. Ag-NPs have been synthesized from different microalgae and macroalgae. Silver-NPs have been known to be used for numerous applications including antimicrobial agent [20]. The development of silver-NPs is highly attractive to researchers due to the nobility of this metal and its wide variety of applications, especially in biomedical and biochemistry fields. The mechanism of Ag-NPs by the algal cells may be owing to the presence of metabolites that reduce silver ions into Ag-NPs including enzymes [21].

This review focused on silver nanoparticle (Ag-NPs) synthesis using microalga and their biological activities. A systematic search was carried out in Pub Med, Scopus and Web of Sciences using a combination of Boolean operators. Peer reviewed papers in English on the keyword silver nanoparticle synthesis by microalgae were retrieved and evaluated based

on titles and abstracts. The retrieved papers were managed using Mendeley and the data were consolidated.

SILVER NANOPARTICLES (AG-NPS) SYNTHESIS USING MICROALGAE

Xie et al., [22] used the extract of economically important unicellular green alga *Chlorella vulgaris*, for the synthesis of silver nanoplates. Vivek et al., [23] obtained spherical Ag-NPs of an average size of 22 nm using the aqueous extract of the red alga *Gelidiella acerosa*. Ag-NPs present in the filtrate were well distributed as non-aggregates and showed a broad λ_{max} peak at 408 nm. Tsibakhashvili et al., [24] carried out extracellular synthesis via *Spirulina platensis* and studied the effect of short term and long term exposure of Ag ions along with its dependence on concentration. Barwal et al., [25] reported in vitro and in vivo biosynthesis of rounded and rectangular Ag-NPs from *Chlamydomonas reinhardtii*. In vitro synthesis was found to be slower, taking 13 days, and so-formed NPs possessed size in the range of 5 ± 1 to 15 ± 2 nm, while in vivo synthesis was a comparatively faster process which took 10 h, and the NPs produced were in the range of 5 ± 1 to 35 ± 5 nm. Crystallized silver nanoparticles (SNPs) have been biosynthesized by *Spirulina platensis* in an aqueous system. An aqueous solution of silver ions was treated with a live biomass of *Spirulina platensis* for the formation of SNPs. The synthesized SNPs had an average size of 11.6 nm [26].

During synthesis of Ag-NPs, chromatic changes in the reaction mixture act as a visual marker affirming the continuity of the process. Kannan et al., [27] observed an obvious change of brown to yellow colour after 48 h during reduction of $AgNO_3$ by the extract of *Codium capitatum* and a time-dependent increase in brown colour intensity at 422 nm. Moreover during reduction of $AgNO_3$ by *Chaetomorpha linum* extract, the same colour change was observed within 30 minutes and with the increase in incubation time, the brown colour intensity decreased at 422 nm viz characteristic absorption peak of Ag-NPs [28]. Kumar et al., [29] successfully fabricated spherical Ag-NPs with an average size of 48.59 nm at room temperature within 48 h of incubation using *Ulva lactuca*. Prasad et al., [30] employed *Cystophora moniliformis* for the synthesis of Ag-NPs. Effect of temperature on the size and agglomeration showed that at temperatures lower than 65 °C, spherical Ag-NPs with size range 50-100 nm and higher temperatures up to 95 °C, NPs of size greater than 2 μm were formed. The NPs so formed were of crystalline nature with FCC geometry as suggested by XRD pattern. Madhiyazhagan et al., [31] reported the synthesis of crystalline spherical Ag-NPs with FCC geometry, ranging from 43 to 79 nm in size using the aqueous extract of *Sargassum muticum*. The synthesis of silver nanospheres was confirmed through visual assessment as the colour of the solution turned from yellowish light brown to dark brown after the addition of 1mM $AgNO_3$ to 5% (w/v) algal extract at 95°C.

Other green algal species like *Nannochloropsis oculata* [19], *Chlorococcum humicola* [32], *Euglena gracilis* [33], *Scenedesmus* sp. [34] etc. have been reported to synthesize Ag-NPs with variable shapes and applications. *Chlorococcum humicola* was exploited for intracellular and extracellular biosynthesis of Ag-NPs using fresh extracts (*in vitro*) and whole cells (*in vivo*) [32]. After incubation of algal extract and whole cells with $AgNO_3$ (5 mM) solution for 48 h at 28 °C, spherical, crystalline Ag-NPs ranging from 2 to 16 nm with face-centered cubic geometry were obtained. Cyanobacterial mediated synthesis of Ag-NPs at large scale was conducted by

Sharma et al., [12]. Li et al., [35] reported in vitro and in vivo biosynthesis of Ag-NPs from *Euglena* spp. and found that the decreased concentrations of silver ions in the solutions, which were treated with *Euglena gracilis* and *Euglena intermedia* were almost equal. Cell free aqueous extract of *Microchaete NCCU-342* was exposed to various cultural and physical conditions for optimizing synthesis of Ag-NPs. Optimal synthesis of Ag-NPs was obtained with biomass quantity of 80 $\mu g/ml$ at pH 5.5 and 60°C with UV light exposure (60 min) and 1mM $AgNO_3$ [36].

BIOLOGICAL ACTIVITIES OF MICROALGAE SILVER NANOPARTICLES (AG-NPS)

The antibacterial activity of silver nanoparticles synthesized using macroalgae *Spirogyra varians* was evaluated by Salari et al., [37]. The antibacterial effect on *B. cereus*, *P. aeruginosa* and *Klebsiella* was more significant compared to standard antibiotic. The mechanism of the bactericidal effect of nanoparticles could be due to SNPs after penetration into the bacteria can inactivate their enzymes, generate hydrogen peroxide and cause bacterial cell death [38]. The antibacterial activity of the silver nanoparticles may be centred on permeability of bacterial cells due to cell wall layers or its charges [39, 40].

In vitro antimicrobial activity of the synthesized nanoparticles of *Enteromorpha flexuosa* exhibited high antibacterial activity against Gram-positive bacteria and low activity against the Gram-negative organisms [41]. Biological synthesis of silver nanoparticles using the cell-free extract of *Spirulina platensis* and its antimicrobial activity was studied by Sharma et al., [12]. The AgNPs had shown maximum zone of inhibition against *P. vulgaris* (31.3 ± 1.11 mm). Aqueous extract of *Spirulina platensis* was used to synthesize AgNPs and evaluated for their antimicrobial activity against isolates obtained from HIV patients [42]. It was observed that *Staphylococcus sciuri* and *P. aeruginosa* are highly susceptible to the antibacterial action of AgNPs with a zone of inhibition of 19 mm followed by *E. coli* with 17.5 mm respectively in the presence of 150 $\mu g/ml$ AgNPs. Antibacterial activity of Ag-NPs synthesized by cyanobacterial (*Anabaena* sp, *Lyngbya* sp, *Synechococcus* sp, and *Synechocystis* sp. *Cylindrospermopsis* sp.) and green algae strains (*Botryococcus* sp., and *Coelastrum* sp.) was tested against bacterial strains for their antibacterial activity [43]. The antibacterial activity of *Pithophora oedogonia* mediated Ag-NPs exhibited potential inhibitory activity against *P. aeruginosa* and *E. coli* [44]. Antimicrobial activity against *Bacillus subtilis* and *Aspergillus flavus* by the red alga, *Laurencia papillosa* synthesized silver nanoparticles was reported by Omar et al., [45]. The green synthesized AgNPs using *Spirulina platensis* were studied its bactericidal activity against *Staphylococcus* sp. and *Klebsiella* sp by Muthusamy et al., [46]. The results indicated that the increasing concentration of AgNPs effectively encountered the bacterial population. El-Kassas et al., [47] reported that Ag-NPs biosynthesized by *T. tetraele* cultures and *H. stipulacea* aqueous extract exerted outstanding negative impacts on *O. simplicissima* in terms of optical density and total chlorophyll. In another study, Ag-NPs from *Oscillatoria limnetica* exhibited strong antibacterial activity against multidrug-resistant *E.coli* and *B. cereus* as well as cytotoxic effects against both human breast (MCF-7) cell line giving IC50 (6.147 $\mu g/ml$) and human colon cancer (HCT-116) cell line giving IC50 (5.369 $\mu g/ml$) [48].

Table-1: Microalgae mediated silver nanoparticle synthesis and their biological activity

Microalgae	Size and Morphology	Biological activity	Reference
<i>Acanthophora specifera</i>	33–81 nm, cubic	Antimicrobial activity against <i>S.aureus</i> , <i>B.subtilis</i> ; <i>Salmonella</i> sp., <i>E.coli</i> , <i>C.albicans</i>	[49]
<i>Acanthophora spicifera</i>	48 nm, spherical	Antimicrobial activity against biofilm forming bacteria <i>S. typhi</i> and <i>S. flexneri</i>	[50]
<i>Amphiroa fragilissima</i>	Crystalline	Antibacterial activity against <i>B.subtilis</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>K. pneumonia</i> , <i>P. aeruginosa</i>	[51]
<i>Caulerpa racemosa</i>	5–25 nm, 10 nm, face-centered cubic	Antibacterial activity against <i>S.aureus</i> , <i>P mirabilis</i>	[52]
<i>Caulerpa serrulata</i>	10±2 nm, spherical, face-centered cubic structure	Antibacterial activity against <i>S. aureus</i> , <i>Salmonella typhi</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>Shigella</i>	[17]
<i>Chlorella pyrenoidosa</i>	5–20 nm with average 12 nm, face-centered cubic	Antibacterial activity against <i>K.pneumoniae</i> , <i>A.hydrophila</i> , <i>Acenetobacter</i> sp, <i>S. aureus</i>	[53]
<i>Chlorella vulgaris</i>	5–50 nm, face-centered cubic	Antibacterial activity against <i>E.coli</i> , <i>P.aeruginosa</i> , <i>Candida albicans</i>	[54]
<i>Chlorococcum humicola</i>	16 nm, spherical	Antibacterial activity against <i>E.coli</i>	[34]
<i>Colpomenia sinusa</i>	20 nm, spherical	Antibacterial activity against <i>S.aureus</i> , <i>E.coli</i>	[55]
<i>Enteromorpha flexuosa</i>	15±1.5 nm, circular	Antimicrobial activity against <i>B.subtilis</i> , <i>S.aureus</i> , <i>E.faecalis</i> , <i>S.epidermidis</i> , <i>E.coli</i> , <i>K.pneumonia</i> , <i>C.albicans</i> , <i>S.cerevisiae</i>	[41]
<i>Euglena gracilis</i>	47 nm	Antibacterial activity against <i>E.coli</i>	[35]
<i>Gelidiella acerosa</i>	22 nm, spherical, face-centered cubic	Antifungal against <i>Humicola insolens</i> , <i>Fusarium dimerum</i> , <i>Mucor indicus</i> , <i>Trichoderma reesei</i>	[23]
<i>Gelidiella</i> sp.	40–50 nm, spherical	Anticancer against Hep 2 cell lines	[56]
<i>Gracilaria birdiae</i>	20.3 nm, spherical	Antibacterial activity against <i>E.coli</i>	[57]
<i>Gracilaria corticata</i>	18–46 nm	Antimicrobial activity against <i>C. albicans</i> and <i>C. glabrata</i>	[58]
<i>Gracilaria dura</i>	6.0 ± 2 nm, sphere	Antibacterial activity against <i>B.pumilus</i>	[59]
<i>Gracilaria edulis</i>	55–99 nm, face-centered cubic, spherical,	Anticancerous against Human PC3 cell lines	[60]
<i>Jania rubins</i>	12 nm, spherical	Antibacterial activity against <i>S.aureus</i> , <i>E.coli</i>	[55]
<i>Nannochloropsis oculata</i> and <i>Tetraselmis tetrathele</i>	13.0-25.2 nm, spherical	Anti-algal activity against <i>Oscillatoria simplicissima</i>	[47]
<i>Oscillatoria limnetica</i>	3.30–17.97 nm, quasi-spherical	Antibacterial activity against <i>E.coli</i> and <i>B. cereus</i>	[48]
<i>Padina gymnospora</i>	25-40 nm, spherical	Antibacterial activity against <i>B.cereus</i>	[61]
<i>Padina pavonica</i>	10-72 nm, spherical, polydisperse	Antifungal activity against <i>Fusarium oxysporum</i> , <i>Xanthomonas campestris</i>	[62]
<i>Padina tetrastromatica</i>	14 nm, spherical	Antibacterial activity against <i>Bacillus</i> spp, <i>B.subtilis</i> , <i>Klebsiella planticola</i> , <i>Pseudomonas</i> sp	[63]
<i>Pithophora oedogonia</i>	25–44 nm, cubical and hexagonal-shaped	Antibacterial activity against <i>E.coli</i> , <i>P.aeruginosa</i> , <i>V.cholera</i> , <i>Shigella flexneri</i> , <i>B.subtilis</i> , <i>S.aureus</i> , <i>Micrococcus luteus</i>	[44]
<i>Pterocladia capillacae</i> ,	7 nm, spherical	Antibacterial activity against <i>S.aureus</i> , <i>E.coli</i>	[55]
<i>Sargassum cinereum</i>	45 to 76 nm, triangular	Antibacterial activity against <i>S.aureus</i> , <i>S.typhi</i> , <i>E.aerogenes</i> , <i>P. vulgaris</i>	[64]
<i>Sargassum ilicifolium</i>	33–40 nm, spherical	Antibacterial activity against <i>S.aureus</i> , <i>E.coli</i> , <i>K.pneumoniae</i> , <i>S.typhi</i> , <i>Vibrio cholera</i> ; Cytotoxic against <i>Artemia salina</i>	[65]
<i>Sargassum longifolium</i>	30 nm, cubical	Anticancer against Hep 2 cell line	[66]
<i>Sargassum longifolium</i>	40–85 nm, spherical, face-centered cubic	<i>A.fumigatus</i> , <i>C.albicans</i> , <i>Fusarium</i> sp. <i>S.longifolium</i>	[67]
<i>Sargassum muticum</i>	5–15 nm, spherical	Antifungal, antiviral, antiplatelet, antiangiogenesis	[68]
<i>Sargassum muticum</i>	43–79 nm, spherical, crystalline, face-centered cubic	Ovicidal and ovideterrent activity against <i>Aedes aegypti</i> , <i>Anopheles stephensi</i> , and <i>Culex quinquefasciatus</i> Antibacterial activity against <i>B. subtilis</i> , <i>S. typhi</i> , <i>K. pneumonia</i>	[32]
<i>Sargassum polycystum</i>	5–7 nm, spherical, face-centered cubic	Antibacterial activity against <i>S.aureus</i> , <i>E.coli</i> , <i>P.aeruginosa</i> , <i>K.pneumonia</i> Anticancer against MCF-7 breast cancer cell lines	[69]
<i>Sargassum polycystum</i>	-	Antibacterial activity against <i>E.coli</i> , <i>Streptococcus pyogenes</i> , <i>P.aeruginosa</i> , <i>S.flexneri</i> , <i>Morengilla morrgani</i> ; Cytotoxic activity: Dalton's lymphoma ascites (DLA)	[70]
<i>Sargassum vulgare</i>	10 nm, spherical	Anticancer: Human myeloblastic leukemic cells HL60, cervical cancer cells HeLa	[71]
<i>Sargassum wightii</i> G revilli	8–27 nm, spherical,	Antibacterial activity against <i>S. aureus</i> , <i>B. rhizoids</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	[72]
<i>Scaberia agardhii</i>	40–50 nm, polydispersed	Antibacterial activity against Soil microbial	[73]

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<i>Scenedesmus sp.</i>	36 nm, spherical, face-centered cubic	Antibacterial activity against <i>S. mutans</i> , <i>E. coli</i>	[74]
<i>Spirogyra</i>	40-80 nm, spherical	Antibacterial activity against <i>S. aureus</i> , <i>E. coli</i>	[75]
<i>Spirogyra varians</i>	17.6 nm, face-centered cubic structure, quasi-spheres	Antibacterial activity against <i>S. aureus</i> , <i>B. cereus</i> , <i>L. Monocytogenes</i> , <i>S. typhimurium</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>Klebsiella</i>	[76]
<i>Turbinaria conoides</i>	96 nm, spherical	Antibacterial activity against <i>B. subtilis</i> , <i>K. planticola</i>	[77]
<i>Turbinaria conoides</i>	2-17 nm, spherical, face-centered cubic	Antibacterial activity against <i>E.coli</i> , <i>Salmonella sp.</i> , <i>S. liquefaciens</i> , <i>A.hydrophila</i> Cytotoxicity and anticrustacean activity: <i>Artemia salina</i>	[78]
<i>Turbinaria ornata</i>	22 nm, spherical, polydisperse	Antibacterial activity against <i>B. litoralis</i> , <i>Bacillus sp.</i> , <i>Micrococcus sp.</i> , <i>Corynebacterium sp.</i> , <i>S.aureus</i> , <i>Flavobacterium sp.</i> , <i>Pseudomonas sp.</i> , <i>Shigella sp.</i> , <i>Aeromonas sp.</i> , <i>V.cholerae</i> , <i>E.coli</i> , <i>Salmonella sp.</i> , <i>E.aerogens</i> , <i>Klebsiella sp.</i> , <i>Chromohalobacter sp.</i> , <i>Artemia marina</i> , <i>Balanus Amphitrite</i>	[79]
<i>Ulva faciata</i>	7-20 nm, spherical	Antimicrobial activity	[55]
<i>Ulva fasciata</i>	28-41 nm, spherical	Antibacterial activity against <i>Xanthomonas campestris</i> pv. <i>Malvacearum</i>	[80]
<i>Ulva lactuca</i>	20-56 nm, spherical	Anticancer activity: Hep2, MCF7 and HT29 cancer cell lines	[81]
<i>Ulva lactuca</i>	20 nm, spherical	<i>Bacillus sp.</i> , <i>Pseudomonas sp.</i> , <i>E.coli</i>	[82]
<i>Ulva lactuca</i>	20-50 nm, spherical	Antimicrobial activity against <i>Bacillus sp.</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>K. pneumonia</i> , <i>P. aeruginosa</i> , <i>C. albicans</i> , <i>A. niger</i> , <i>C. parapsilosis</i>	[83]
<i>Ulva lactuca</i>	20-35 nm, cubical, face-centered cubic	Control of malarial plasmids. <i>P. falciparum</i>	[84]
<i>Urospora sp</i>	20-30 nm, face-centered cubic, spherical	Antibacterial activity against <i>S. aureus</i> , <i>B. subtilis</i> ; <i>E.coli</i> , <i>P.aeruginosa</i> , <i>K. pneumonia</i>	[85]

CONCLUSION

Nano-biotechnology is gaining attention due to its eco-friendly, economical and green approach to silver nanoparticle synthesis. The natural products involving in the synthesis of AgNPs received tremendous attention in the field of bio-nanomaterials. Microalgae have been explored to synthesize Ag-NPs with biological activities. Emerging advanced characterization techniques would facilitate comparative and controlled performance of NPs, which will encourage judicious selection of algae-based NPs. Based on available reports presented in this review, in the future, a remarkable boom may be witnessed in the biosynthesis of algae-based nanoparticles that will be likely to have potential application of antibacterial agent against pathogens and other biological activities.

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