Microalgae mediated synthesis of silver nanoparticles and their antibacterial activity against pathogenic bacteria

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Silver nanoparticles is known to have antimicrobial affects. Cyanobacteria isolates from muthupet mangrove includes Aphanothece sp, Oscillatoria sp, Microcoleus sp, Aphanocapsa sp, Phormidium sp, Lyngbya sp, Gleocapsa sp, Synechococcus sp, Spirulina sp with were set in compliance with their cellular mechanism of nano silver creation, and were investigated by UV-VIS spectrophotometer, Energy-dispersive X-ray (EDX) and scanning electron microscopy (SEM). Silver nanoparticles were spherical shaped well distributed without aggregation in solution with an average size of about 40- 80 nm. Synthesised nano silver had antibacterial production on various organisms that provoked various diseases in humans. The cellular metabolites of Microcoleus sp. only created nano silver and it enhanced the antibacterial activity against test pathogenic bacteria from MTCC (Proteus vulgaris, Salmonella typhi, Vibrio cholera, Streptococcus sp., Bacillus subtilis, Staphylococcus aureus, Escherichia coli.) The antimicrobial assay was performed using 0.001 M concentration of nano silver in well diffusion method with positive control of appropriate standard antibiotic discs Cephotaxime, Ampicillin, Tetracyclin, Cephalexin etc. Synthesised silver nanoparticles acted as an effective antimicrobial agent and proved as an alternative for the development of new antimicrobial agents to combat the problem of resistance.

Keywords: Antimicrobial agents, Cyanobacteria, Microcoleus sp., Resistance, Silver nanoparticles

The development of reliable experimental protocols for the synthesis of nanomaterials over a range of chemical compositions, sizes, and high monodispersity is one of the challenging issues in current nanotechnology. A novel biological method for synthesis of silver nanoparticles using Vericillum proposed by a two-step mechanism was suggested; the first step involves trapping of Ag+ ions at the surface of the biological cells and in the second step, enzymes present in the cell reduce silver ions and becomes nanosilver particles.

Silver nanoparticles comprise a great number of applications including high-sensitivity biomolecular detection, diagnostics, antimicrobial activity, therapeutics and catalysis etc. In the past few decades, the emergence of multi-drug resistant bacterial strains of clinically important pathogens has stimulated the interest of scientists to develop newer broad spectrum of antimicrobial agents.

Marine microalgae are rich source of structurally novel and biologically active metabolites. These microalgae may produce primary or secondary metabolites which are potentially bioactive compounds of interest in pharmaceutical industries. Nowadays, several chemically distinctive compounds of marine origin with a variety of biological activities have been isolated, a few of them are under investigation and are being used to develop new pharmaceutical products. The active constituents of different algal extracts have shown antimicrobial activity against various pathogenic bacteria.

Earlier reports revealed that algal mediated synthesis of nanoparticles has been unexplored and underexploited. Recently few of the researchers reported that microalgae have been used for the production of metallic nanoparticles. Many biological sources like Sargassum wightii, Kappaphycus alvarezi and Gelidiella acerosa crude extracts used for nanometallic conversion. Spirulina platensis was reported to be used for synthesis of silver nanoparticles.

The marine microalgae showed rapid synthesis of silver nanoparticles with high efficacy of antibacterial activity against human pathogens including Escherichia coli, Klebsiella sp, Proteus vulgaris and Pseudomonas aeruginosa.

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It is proved that biological active substances have been extracted from microalgae. Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity. These metabolites influencing the antimicrobial agent production depends various important factors such as temperature of incubation, pH of the culture medium, incubation period, medium constituents and light intensity.17

Specially formulated metal oxide nanoparticles have good antimicrobial activity. However, as the microbial synthesis of nanoparticles strongly depends on the experimental conditions, further investigations are necessary.

The aim of this study is to isolate cyanobacteria from mangrove forest and test the ability of synthesising silver nanoparticles from cellular extracts of cyanobacterial isolates including Aphanothece sp, Oscillatoria sp, Microcoleus sp, Aphanocapsa sp, Phormidium sp, Lyngbya sp, Gloeocapsa sp, Synechococcus sp and Spirulina sp, a novel strains used for nano conversion; and also characterise the size of synthesised silver nanoparticles and study their antimicrobial property against various pathogenic bacteria.

Materials and Methods

Culture and growth conditions—Microalgal water samples were collected from mangrove forest and were isolated and purified by microbiological methods of serial dilution. Based on microscopic morphological appearance cyanobacterial isolates were identified as Aphanothece sp, Oscillatoria sp, Microcoleus sp, Aphanocapsa sp, Phormidium sp, Lyngbya sp, Gloeocapsa sp, Synechococcus sp and Spirulina sp. These isolated micro algae were cultured in ASN III medium under ≤ 3000 lux light intensity with static condition, for 16 h under illumination and 8 h under darkness. Cyanobacterial cultures were harvested approximately after a production period of 15 days.

Bacterial test pathogens—Proteus vulgaris (MTCC 426), Salmonella typhi (MTCC 98), Vibrio cholera (MTCC 3905), Streptococcus sp (MTCC 389), Bacillus subtilis (MTCC 2393), Staphylococcus aureus (MTCC 3160), E.coli (MTCC 4604) and Corynebacterium sp (MTCC 3080) were procured from the Microbial Type Culture Collection (MTCC) center, CSIR-IMTECH, Chandigarh, India. These cultures were grown on appropriate medium at 37 °C for overnight incubation and maintained at 4 °C in a refrigerator.

Biosynthesis of silver nanoparticles from microalgae (MaSNPs)—The culture of microalgal strains growing in ASN III were harvested by centrifugation and these pellets were washed with sterile distilled water to remove the traces of media salts. The cell filtrate and biomass of 5 ml were treated with 95ml of AgNO3 solution (1 mM) and incubated at room temperature for 24 h. During the incubation period change in colour from pale yellow to brown indicates nanoparticle synthesis. Capping of microalgal proteins metabolites and reduction of silver ions may lead to the formation of silver nanoparticles in the solution.

Description of MaSNPs—Preliminary detection of MaSNPs was done by visual observation of color change in cellular extracts. These were recorded on Shimadzu UV-1601 spectrophotometer containing double beam in identical compartments each for reference and test solution fitted with 1cm path length quartz curettes. Energy-dispersive X-ray spectroscopy analysis for the confirmation of elemental silver was carried out for the detection of elemental silver. Purified MaSNPs in suspension were characterized for their size using scanning electron microscope (SEM) (JEOL-JSM 6390, Japan). Energy dispersive X-ray analysis of purified MaSNPs was carried out using the same instrument for confirming the elemental composition of the sample.

Antimicrobial assay of MaSNPs—In order to study the antibacterial efficiency of Microalgae mediated synthesis of silver nanoparticles by agar well diffusion method, it was set against Corynebacterium sp. (MTCC 3080), P. vulgaris (MTCC 426), S.typhi (MTCC 98), V.cholera (MTCC 3905), Streptococcus sp (MTCC 389), B.subtilis (MTCC 2393), S.aureus (MTCC 3160) and E.coli (MTCC 4604). The pure cultures of bacterial pathogens were sub-cultured on appropriate medium. Wells of 5 mm diameter were made on Muller Hinton agar and blood agar plates using gel puncture. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette, different concentrations of the sample of nanoparticles solution (10, 20 and 50 µl) were poured into each well on all plates. After incubation at 37 °C for 24 h, the different levels of zones of inhibition of bacteria were measured. The assays were performed in triplicate.
Results and Discussion

*Microcoleus mediated reduction of silver ions*—The cyanobacterial biomass and filtrate after addition of aqueous AgNO\(_3\) (1 mM) in the dark, samples changed in colour from almost colourless to brown, with intensity increasing during the period of incubation (Fig. 1). Formation of colloidal silver nanoparticles can be easily followed by changes of UV-Vis absorption (Fig. 2). Among the collected microalgae, *Microcoleus sp* biomass and AgNO\(_3\) reactive mixture only synthesised silver nanoparticles. The mechanism of action of Ag nanoparticles synthesis was not known, but later it was hypothesized that the silver ions required the NADH-dependent nitrate reductase enzyme for their reduction\(^{19-21}\). Shankar suggested that the shoulder at 370 nm corresponded to the transverse plasmon vibration in silver nanoparticles, whereas the peak at 440 nm due to excitation of longitudinal plasmon vibrations\(^{22}\). After 72 h, the process was stopped and the particles were further analyzed by scanning electron microscopy and EDX.

Fig. 1 represents the periodical colour changes visually during the interaction of Ag and microalgae. Fig. 2 depicts a series of typical UV-Vis spectra of the reaction solution recorded at intervals of 24h. Under normal pH 6.0, the change in light absorption profile of the medium and the change in intensity of the brown colour during long term incubation (72 h), showed an increased absorbance with increasing time of incubation at characteristic surface Plasmon resonance absorption band indicative of relatively smaller monodisperse and spherical silver particles.

*Characterisation of MaSNPs*—Scanning electron microscopic analysis was carried out to understand the topology and the size of the MaSNPs, Fig. 3 showed the synthesis of polydisperse spherical Ag-NPs in the range 44-79 nm with average size of 55 nm. The energy dispersive X-ray spectroscopy analysis of the MaSNPs confirmed the elemental composition of nanoparticles as silver by sharp signals (Fig. 4).

![Fig. 1—Periodical colour change in the bacterial mediated extra cellular nanoparticles [a: 0 h, b: 1st h, c: 2nd h, d: 4th h, e: 6th h, f: 24th h]](image)

![Fig. 2—UV–Vis spectra of algal extract containing silver nanoparticles.]](image)
**Fig. 3**—Scanning electron micrograph of aggregated and protein-capped silver nanoparticles and spherical silver nanoparticles (44–64 nm).

**Fig. 4**—EDX analysis of the MaSNPs confirmed the elemental composition of nanoparticles as silver.
The optical absorption band peak in the range of 3-4 keV is typical for the absorption of metallic silver nanocrystallites\textsuperscript{23}.

**Antimicrobial activity of MaSNPs**—The results obtained from the present study concerning the biological activity of the antimicrobial agents produced by *Microcoleus* sp synthesised silver nanoparticles against different species of bacteria were recorded (Table 1, Fig. 5). The diameter of the inhibition zone depends mainly on the type of the algal species, silver nanoparticles and the test bacterial organisms. Here the efficiency of antimicrobial property of *Microcoleus* sp synthesised silver nanoparticles when compared to standard antibiotics can be justified. Even the microalgal synthesised silver nanoparticles are partially purified crude particles, when go for further purification it will show more significant effect than a commercial antibiotic. Concerning antibacterial effects, the results cleared that *Microcoleus* sp gave the highest biological activities when compared to the remaining microalgae and were found to synthesise silver nanoparticles and produce bactericidal effects against test bacterial strains. These results go in harmony with those obtained by Volk et al\textsuperscript{24}. They found that some microalgae had high biological activity against *B. subtilis*, *B. megaterium*, *E. coli*, etc\textsuperscript{24}. Anent the MaSNPs of the microalgae *Microcoleus* sp, (Fig. 6) the results revealed that the antibacterial effect was greater towards *E. coli* (11 mm), *P. vulgaris* (12 mm), *B. subtilis* (12 mm), *Corynebacterium* sp (12 mm) and *S. aureus* (11 mm). Minor antibacterial effect was recorded towards the lateral bacterial species. Concerning the silver nanoparticle synthesising and antibacterial effects of the tested green microalgae, the result recorded in Table 1 cleared that biomass of *Microcoleus* sp synthesises silver nanoparticles had the highest antibacterial activity against *E. coli*, *P. vulgaris*, *B. subtilis* and *Corynebacterium* sp. moderate activity against *S. aureus* and a weak activity towards *V. cholerae* and *S. typhi*. At the same time, apart from *Microcoleus* sp, no other microalgae synthesised silver nanoparticles. The extracts of 12 cyanobacterial strains were investigated for their antibiotic activities against 7 microorganisms by Kreitlow et al\textsuperscript{25}.

To date, biomass synthesis of silver nanoparticles by *Microcoleus* sp has not been reported and this has been reported in this communication for the first time. Moreover, the silver nanoparticle synthesised from *Microcoleus* sp and its antimicrobial activity against

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<tr>
<th>Test bacteria</th>
<th>Standard antibiotics</th>
<th>MaSNPs</th>
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<td><em>E. coli</em></td>
<td>15 (Cf)</td>
<td>11</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>14 (Ce)</td>
<td>12</td>
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<tr>
<td><em>S. typhi</em></td>
<td>15 (A)</td>
<td>9</td>
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<tr>
<td><em>V. cholerae</em></td>
<td>16 (T)</td>
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<td><em>B. subtilis</em></td>
<td>20 (T)</td>
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<tr>
<td><em>S. aureus</em></td>
<td>16 (Cf)</td>
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<td><em>Streptococcus</em> sp</td>
<td>15 (A)</td>
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<tr>
<td><em>Corynebacterium</em> sp</td>
<td>14 (cf)</td>
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Fig. 5—Graphical representation of antimicrobial effect of MaSNPS with bacteria and standard antibiotics.
human pathogens is similarly a new finding. Finally it is concluded that microalgae are potential sources of bioactive compounds and should be investigated for natural antibiotics; however, further work is required to identify these active compounds.

Conclusions

It is concluded that among the collected nine different genera of mangrove vegetation microalgae, Microcoleus sp synthesised silver nanoparticles. In this analysis, the MaSNPs showed an excellent antimicrobial activity against all the eight test pathogens when compared to normal Ag. Microbes mediated AgNPs. They also revealed high antimicrobial activity and were eco-friendly, economically low cost, renewable etc. Subsequently, these marine sources are a future vision for novel antimicrobial agents.

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References