Antibacterial and anticancerous biocompatible silver nanoparticles synthesised from the cold-tolerant strain of *Spirulina platensis*

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**Objective:** To synthesize silver nanoparticles from the biomass of cold tolerant strain of *Spirulina platensis* and evaluate the synthesized nanoparticles against antibacterial and anticancer activity.

**Methods:** Silver nanoparticles were synthesized by the algal culture and characterized by UV-vis spectroscopy, Fourier transform infrared spectroscopy, field emission scanning electron microscopy and X ray diffraction studies. Antibacterial activity has been studied with free nanoparticles adopting agar diffusion assay, biofilm inhibition assay and nanoparticles fabricated wound dressing against representative Gram-negative organism *Pseudomonas aeruginosa* and Gram-positive organism *Staphylococcus aureus* respectively. The in vitro anticancer activity of silver nanoparticles were screened against human Hep2 cell lines by means of MTT assay.

**Results:** Reduction of silver ions by the algal culture was observed during 72 h of incubation and the synthesized nanoparticles were further characterized. Antibacterial study reveals both the strains were susceptible to free nanoparticles and fabricated wound dressing treatment. The in vitro anticancer activity of silver nanoparticles were screened against human Hep 2 cell lines by means of MTT assay which reveals that cell viability has been reduced as dose dependent manner.

**Conclusions:** The observed results imply that silver nanoparticles synthesized from *Spirulina platensis* cold tolerant strain can be used as potential antibacterial and anticancerous agent.

**KEYWORDS**

*Spirulina platensis*, Silver nanoparticles, Antibacterial, Anticancerous
vehicles and are currently used in gene and drug delivery as well as in cancer diagnostics and therapeutic applications[4].

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. The application of nanoparticles as delivery vehicles for bactericidal agents represents a new paradigm in the design of antibacterial therapeutics[5,6]. Silver nanoparticles commonly used for nanomedicine production, are reported to be nontoxic to human, but most effective against bacteria, viruses, and other eukaryotic microorganisms at very low concentrations[7]. They are also effective against tumors with anti-proliferative activity[8]. The antimicrobial property allows them to be suitably employed in numerous products such as textiles, food storage containers, home appliances and especially in medical devices[9]. Use of silver nanoparticle is in medicine industry as topical ointments to prevent infection against burn and open wounds is quite effective. Silver nanoparticles play important role as pesticide filter also[10]. The development of biologically inspired experimental process for synthesis of nanoparticles is evolving into an important branch of nanotechnology[11,12]. Biologically, synthesized silver nanoparticles could have many applications: they might be used as spectrally-selective coatings for solar energy absorption and intercalation material for electrical batteries; they also begin to be used as optical receptors and as catalysts in chemical reactions. Concerning the biological application of nanoparticles it has been emphasized that methods of synthesis through biological systems viz. microorganisms including bacteria, yeasts, fungi and diatoms synthesizing inorganic materials either intra or extracellularly would make the nanoparticles more biocompatible. Different plant extracts have been used and reported for synthesis of gold, silver and bimetallic nanoparticles[13,14].

Silver nanoparticles are synthesized by different physical and chemical methods like sol-gel technique, solvo-thermal synthesis, chemical reduction, laser ablation, inert gas condensation, etc. The time consuming physical methods are often difficult to achieve and in chemical methods different toxic reagents are used as capping agent like, cetyltrimethyl ammonium bromide leading to undesirable functional aberrations in target cells. Both physical and chemical procedures are very expensive. Therefore, biosynthesis of silver nanoparticles are becoming popular day by day using microorganisms like bacteria[15-18], fungi[19-21]. A algal mediated synthesis of silver nanoparticles is now being extensively carried out by researchers because of the high rate of synthesis, efficacy and best compatibility[22]. Synthesis of biocompatible potential bioactive silver nanoparticles from various algal crude extracts of Sargassum wightii, Kappaphycus alvarezzii, Gelididdela acerosa[23-25], Spirulina platensis (S. platensis) [26], Pterocladia capillaceae, Jania rubins, Ulva fasciata, and Colpmenia sinus[27]. Potential antimicrobial silver nanoparticles synthesized from marine microalgae against human pathogenic bacteria has been reported[28]. In the present study, antibacterial and anticancerous silver nanoparticles synthesized from cold resistance strain of S. platensis has been studied.

2. Materials and methods

2.1. Algal strain and growth condition

Laboratory stock culture of S. platensis was cultured in BG-11 (Blue-Green algae) medium for cyanobacteria[29]. The growth potential of alga was maintained through regular sub-culturing techniques, under laboratory conditions at 28 °C, in a 16/8 h light/dark cycle, under cool fluorescent light (20-30 μmol photons/m²/s), in BG-11 medium (pH 9). Cold-tolerant strain of S. platensis has been raised from the subculture by the modified method of K im et al[30]. S. platensis was grown at 28 °C to an OD 600 of 0.4, and aliquots were transferred to temperature-equilibrated flasks in water baths at 15 °C. Samples were taken at 30 min after transfer. Then, 5 mL of the cold stressed culture was transferred to the conventional BG-11 media and the culture thus obtained was used for biosynthesis of silver nanoparticles.

2.2. Synthesis of silver nanoparticles

Synthesis of silver nanoparticles by cold-resistant strain of S. platensis was carried out by the modified method of M ahdieh et al[31]. A total of 5 mL of exponential growth phase culture of S. platensis was transferred to the 250 mL Erlenmeyer flask with 100 mL of 1 mmol/L aqueous AgNO₃ solution (pH 7) for 24 h under shaking condition at 28 °C.

2.3. Characterization

UV-vis spectral analysis was performed on a Shimadzu-1800 spectrophotometer. The biosynthesized silver nanoparticles solution was centrifuged at 10000 r/min for 15 min and the suspension was redispersed in sterile distilled water. Finally, dried samples were palladized with KBr for Fourier transform infrared spectroscopy (FTIR) measurements. The spectrum was recorded in the range of 4000-500 cm⁻¹ using Bruker OpticGmbh Tensor 27. X-ray diffraction (XRD) measurement of the silver nanoparticles was carried out using Rigaku smart lab instrument operated at a voltage of 40 kV and a current of 30 mA with Cu Kα1 radiations Field emission scanning electron microscopy-energy dispersive X-ray analysis (FESEM-EDAX) was performed by Supra 55-Carl Zeiss, Germany.

2.4. Evaluation of biological activities

2.4.1. Antibacterial activity

The antibacterial activity of silver nanoparticles was studied against Pseudomonas aeruginosa (ATCC 10145) (P. aeruginosa) and Staphylococcus aureus (ATCC ) (S. aureus) adopting well diffusion assay. Both the strains were obtained from American type collection (ATCC) and maintained on tryptic soy agar slants. A loopful of slant culture was inoculated into tryptic soy broth and incubated at 37 °C for 12-16 h to reach mid log phase. The respective broth culture was uniformly spread with sterile cotton swabs on sterile Mueller Hinton
agar media (Hi-media, India). The wells were made using cork borer and aliquots of silver nanoparticles (aliquots of 25, 50, 75 μg/mL) were prepared from concentrated silver nanoparticles) was loaded into the wells. The plates were incubated at 37°C for 24 h.

2.4.2. Determination of minimum inhibitory concentration (MIC)

Modified method of microdilution calorimetric assay using the chromogenic reagent MTT was used to study the MIC[31]. Respective bacterial strains were grown in Luria-Bertani medium (yeast extract 5 g, peptone 10 g/L, sodium chloride 5 g/L, and pH 7.0) overnight at 28°C. A total of 10 μL of different concentration of nanoparticles(10-100 μg/mL) and prepared bacterial suspension (90 μL) containing 1-10^6 CFU/mL were added into each well of the 96 well microplate. The microtiter plates were incubated in the dark at 28°C for 24 h. Then 10 μL of MTT (5 mg/mL in 0.2 mol/L, pH 7.2, phosphate buffer saline) was added into each well and the plates were incubated another 4 h. The MIC value was defined as the lowest sample concentration that inhibited visible growth of the test bacterium, as indicated by MTT straining. Only living microorganisms can convert MTT to formaldehyde and a blue colour appeared in the well[32](Abe et al., 2000).

2.5. Biofilm inhibition study

2.5.1. Inocula preparation

Respective bacterial culture was inoculated from fresh slopes of tryptic soy agar into tryptic soy broth and incubated with shaking at 37°C for 24 h. Cells were collected by centrifugation and the collected cell debris washed twice in phosphate buffer saline and suspended to OD520 prior to use in biofilm experiments[33].

2.5.2. Biofilm inhibition assay

Biofilm inhibition was studied by the microtiter plate spectrophotometric assay. A total of 100 μL of respective bacterial cell suspension and the respective concentration of silver nanoparticles was added into the wells of a 96-well polyvinyl chloride microtiter plate. The microtiter plates were covered and sealed before incubation under stationary conditions at 37°C for 24 h. After the incubation time, the content was discarded and the plates thoroughly washed with water. Then 100 μL of 0.1% aqueous solution of crystal violet was added and incubated at room temperature for 30 min followed by washing with water. The remaining stain was solubilized with 200 μL of 95% ethanol. Biofilm inhibition was studied by determination of the absorbance of the ethanol solubilised mixture at 540 nm in an UV spectrophotometer. Control (without bacteria only crystal violet), three replicates were maintained for each treatment[34,35].

2.6. Effect of nanoparticles on the biochemical composition of biofilm matrix

2.6.1. Isolation of biofilm matrix

Effect of silver nanoparticles on the biochemical composition of biofilm matrix material was isolated from the microtiter plate was studied by the method of Azeredo and Sutherland[36]. A dherent biofilms were transferred to screw cap bottles containing 10 mL distilled water. The bottles were sonicated for 5 min in an ultrasonic water bath and vortexed vigorously for 1 min to disrupt the biofilms. Cell suspensions were then pooled and centrifuged. The collected supernatant used as source for studying biochemical composition mainly protein by Lowry et al. and total carbohydrate by Dubois et al.[37].

2.6.2. Nano fabrication of silver nanoparticles on the wound dressing

Fabrication of silver nanoparticles on the wound dressing material was carried out by the modified method of Shuangyun et al.[38]. Fabrication was achieved by submerging the dressing pieces (10-10 mm) in 5 mL of silver nano suspension (1 μg/mL) and then the dressing pieces have been extemporaneously dried at room temperature, sterilized by ultraviolet irradiation for 30 min.

2.6.3. Characterization of wound dressing

The surface topography of nanoparticles coated dressing was characterized by scanning electron microscopy.

2.6.4. Anticancer activity

The antibacterial activity of coated dressing was tested against P. aeruginosa and S. aureus adopting agar diffusion assay. Sterilized nano fabricated wound dressing was placed on the M ueller Hinton agar plates swabbed with respective bacterial culture incubated at 37°C for 24 h and the plates were observed for zone of inhibition. After the incubation period the diameter of the zone was recorded.

2.7. Anticancer activity

2.7.1. Chemicals

RPMI1640, fetal bovine serum, trypsin, methylthiazolylidiphenyl-tetrazolium bromide (MTT), and dimethyl sulfoxide were purchased from Hi media & SigmaAldrich Mumbai.

2.7.2. Cytotoxicity assay

Cytotoxicity of silver nanoparticles was determined by inhibition of cell growth of Hep2 cell line using a tetrazolium dye (MTT) assay and percentage of cell viability was determined by spectrophotometric determination of accumulated formazan derivative in treated cells at 570 nm in comparison with the untreated ones. Hep2 cell line was obtained from National centre for cell sciences, Pune, India. RPMI 1640 was used as the source of cell growth medium and a humidified atmosphere (d 5% CO₂) was maintained for cell culture. Hep2 cells were harvested in a logarithmic growth phase, then seeded on 96 wells at a cellular density of 5-10^4 cells/mL followed by the addition of 1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8 μg/mL concentrations of nanoparticles, incubated for 24 h at 5% CO₂ incubator. After removal of the sample solution and
washing with phosphate-buffered saline (pH 7.4), 20 μL/well (5 mg/mL) of 0.5% MTT in phosphate buffered saline solution was added. After 4 h incubation, 1 mL of dimethyl sulfoxide was added. Viable cells were determined by the absorbance at 540 nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The effect of the nanoparticles on the proliferation of Hep 2 cells was expressed as the % cell viability, using the following formula:

\[
\text{Percentage of cytotoxicity} = \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} 
\times 100
\]

3. Results

3.1. Synthesis and characterization of silver nanoparticles

In the present study, silver nanoparticles were synthesized from cold-resistance strain of *S. platensis* and evaluation of antibacterial and anticancerous activities has been made. Reduction of silver ions into silver nanoparticles by the algal culture was visually identified by colour change from yellow to brown in the aqueous solution of reaction mixture at 1 h incubation time and the intensity of the colour increased during the increased incubation time (72 h). UV-vis absorption spectroscopy analysis reveals a broad surface plasmon absorption maxima at 430 nm (Figure 1). Moreover a surface plasmon peak remain in the range of 430 nm at increasing incubation period (72 h) suggesting nanodispersive particles in the aqueous solution. Characterization of synthesized silver nanoparticles by scanning electron microscopy reveals spherical particles with the size of 27 nm (Figure 2). Further, characterization was carried out by FTIR. FTIR spectrum of the synthesized nanoparticles shows strong peak at 3748 cm\(^{-1}\) reveals free alcoholic and carboxylic acid groups present (Figure 3). The peak at 2367 cm\(^{-1}\) designates the asymmetric -CH bending, thus indicating its role in reduction of silver ions. Further, the presence of peak at 1762 cm\(^{-1}\) confirms the amide I stretching group. Peak at 1384 cm\(^{-1}\) revealed C@C responsible for the reduction of silver salt. The formation of synthesized silver nanoparticles was further supported by XRD measurements. This method is used to elucidate crystallinity and the lattice properties of the silver nanoparticles. Presence of distinct high diffraction peaks at 21.6º, 35.5º and 43.5º respectively, indexing the Bragg’s reflection planes (111), (200), (220) and (311) confirmed the face centered cubic structure of crystalline silver nanoparticles (Figure 4).

3.2. Antibacterial activity

In the present study, antibacterial activity was studied against *P. aeruginosa* and *S. aureus*. It can be seen that both the tested strains were susceptible to the silver nanoparticles as dose dependent manner (Table 1). An increase in inhibitory zone was recorded in high concentration (Figures 5a and 5b). In the case of *P. aeruginosa*, maximum zone of inhibition was recorded at 75 μg/mL with 29.0 mm followed by 50 μg/mL with 24.2 mm, 25 μg/mL with 22.0 mm...
of zone of inhibition (Figure 5a) S. aureus shows high sensitivity to high concentration of nanoparticles (75 μg/mL) as in P. aeruginosa. Zone of inhibition against S. aureus (32.1, 28.0 and 22.0 mm) has been observed at the respective concentration (Figure 5b). MIC of the nanoparticles against both the tested bacterial strains was studied by broth dilution method. The MIC values of nanoparticles against P. aeruginosa and S. aureus was found to be 31.2 and 29.2. It can be seen that nanoparticles showed high antibacterial efficacy.

Table 1
Zone of inhibition of silver nanoparticles against pathogenic bacteria.

| Concentration (μg/mL) | Zone of inhibition (mm) |
|-----------------------|-------------------------|
|                       | S. aureus | P. aeruginosa |
| 25                    | 22.0      | 22.0         |
| 50                    | 28.0      | 24.2         |
| 75                    | 32.1      | 29.0         |

Changes in reduction of total carbohydrates and protein have been recorded in nanoparticles treatment as dose dependent manner. Antibacterial activity of nanoparticles fabricated wound dressing has been studied. Surface topography of wound dressing with SEM reveals complete dispersion of nanoparticles on the fiber surface and the size of the embedded particles was 60-70 nm (Figure 7). A nbtobiofilm activity of nanoparticles fabricated wound dressing was studied by solid plate agar diffusion assay. Both the bacterial strains were found to be susceptible. Zone of inhibition 17.0 and 16.5 mm was recorded against P. aeruginosa and S. aureus respectively (Figures 8a and 8b).

Table 2
Effect of silver nanoparticles on the biochemical composition of biofilm matrix of pathogenic bacteria.

| Concentration (μg/mL) | S. aureus | P. aeruginosa | S. aureus | P. aeruginosa |
|-----------------------|-----------|---------------|-----------|---------------|
| 25                    | 203.4     | 105.0         | 231.4     | 198.0         |
| 50                    | 121.5     | 54.0          | 78.0      | 86.7          |
| 75                    | 51.0      | 12.0          | 11.0      | 20.4          |

The in vitro cytotoxicity effects of silver nanoparticles synthesized from S. platensis were screened against human Hep 2 cell lines by means of MTT assay. A serial 10 fold dilution of silver nanoparticles was prepared. Hep 2 cells grown in 96 well plates were incubated and the viability in respective dilution was determined by MTT assay which reveals that nanoparticles reduced the viability as dose dependent manner. Effective high cytotoxic effect was recorded at 1000 μg/mL followed by 500, 250, 125, 62.5, 31.2, 15.6, 7.8 μg/mL.

3.3. Anticancer activity

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and the percentage of viability at respective concentration was 8.7%, 13.5%, 15.5%, 20.3%, 26.2%, 29.1%, 45.6% and 59.2% respectively (Figure 9).

Figure 9. Effect of silver nanoparticles on viability (%) of Hep2 cell lines.

Morphological characteristics of silver nanoparticles treated cells using an inverted microscope revealed that changes in the cell morphology were observed at 1000 and 500 μg/mL when compared to the cells treated at least concentrations (Figure 10). It can be clearly observed that the cells treated with silver nanoparticles with 1000 μg/mL showed distinct changes in the morphological features whereas cells treated with 7.8 μg/mL have a well-developed nucleus, devoid of peripheral cellular distribution as in control cells. The cytotoxic effect of silver nanoparticles on cell viability has a major role in antitumor activity, thereby reducing disease progression. The cytotoxic effects of silver are the result of active physiochemical interaction of silver atoms with functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA[39].

4. Discussion

Nowadays, biological method of nanoparticles synthesis is a vast growing technique in the field of nanotechnology. The biological sources had the more quantity of trouble-free protocols and when applied for the human health associated field, it is easy to approach for maintain aseptic environment during the synthesis process of nanoparticles. Algal mediated synthesis of silver nanoparticles is now being extensively carried out by researchers because of the high rate of synthesis, efficacy and best compatibility[22]. Synthesis of silver nanoparticles from cold resistance strain of S. platensis was primarishly confirmed. The formation of brown colour suggests the presence of silver nanoparticles[19] due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field[40]. Mechanism of synthesis of silver nanoparticles by the algal culture is due to the production of metabolites that reduces silver ions into silver nanoparticles and these metabolites mainly enzymes such as NADH dependent nitrate reductase[41]. Size distribution analysis of the capped silver nano conjugates confirmed that the particles were well dispersed. Bio-organic materials of the algal cells bound to the nanoparticles surface which is responsible for the stability of nanoparticles[42,43]. It is evident through the FTIR spectra that the presence of different functional groups in the algal biomass might serve as the reducing and capping agents of the silver nanoparticles. Further characterization was carried out by XRD. All the peaks in the XRD pattern can be indexed as a standard silver crystal[44] which also shows a high purity as synthesized silver crystal.

Due to the increasing spread of antibiotics or other conventional chemotherapeutics resistance pathogenic strains and cancer cells, it is necessary to develop effective, less toxic biocides. Nanotechnology principles mainly inorganic nanomaterials has been exploited widely in the field of medicine and healthcare worldwide. The common antimicrobial agents are extremely toxic, poor efficacy and it is necessary to formulate new types of safe and cost-effective biocidal materials. Antimicrobial formulations in the form of nanoparticles could be used as potential bactericidal materials. A reactive metal nanoparticles display excellent biocidal action against Gram-positive and Gram-negative bacteria as demonstrated. Thus, the preparation, characterization, surface modification, and functionalization of nanosized inorganic particles open the possibility of formulation of a new generation of bactericidal materials. The improved antibacterial activity could be due to the amount of silver ions released from the nanoparticles which act as reservoirs for the same. The common antimicrobial agents are extremely toxic, poor efficacy and it is necessary to formulate new types of safe and cost-effective biocidal materials. Antimicrobial formulations in the form of nanoparticles could be used as potential bactericidal materials A reactive metal nanoparticles display excellent biocidal action against Gram-positive and Gram-negative bacteria as demonstrated. Thus, the preparation, characterization, surface modification, and functionalization of...
nanosized inorganic particles open the possibility of formulation of a new generation of bactericidal materials. The improved antibacterial activity could be due to the amount of silver ions released from the nanoparticles which act as reservoirs for the same. Antibiofilm effect reveals both the tested strains were found to susceptible and the biochemical composition of the biofilm matrix was highly reduced in nanoparticles treatment. The matrix is one of the most distinctive features of a microbial biofilm. It forms a three dimensional, gel-like, highly hydrated and locally charged environment in which the microorganisms are largely immobilized. Matrix-enclosed micro colonies, sometimes described as stacks or towers, are separated by water channels which provide a mechanism for nutrient circulation within the biofilm. The composition of the matrix varies according to the nature of the organism. Reduction of the biochemical composition of the biofilm matrix leads to weakening of the biofilm thus facilitate entry of the drugs[33]. In the present study, antibacterial and anticancerous silver nanoparticles synthesized from cold resistance strain of S. platensis would suggests the possible utilization of biogenic silver nanoparticles as an effective and biocompatible biocide.

Conflict of interest statement

We declare that we have no conflict of interest.

Background

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. The application of nanoparticles as delivery vehicles for bactericidal agents represents a new paradigm in the design of antibacterial therapeutics. Nanomaterials based on biological origin are highly appreciable because of less toxicity, high efficacy and biocompatibility.

Research frontiers

Silver nanoparticles were synthesized by the algal culture and characterized by UV-vis spectroscopy, FTIR, field emission scanning electron microscopy and XRD studies.

Related reports

Antibacterial activity has been studied with free nanoparticles adopting agar diffusion assay, biofilm inhibition assay and nanoparticles fabricated wound dressing against representative Gram-negative organism P. aeruginosa and Gram-positive organism S. aureus.

Innovations and breakthroughs

The in vivo anticancer activity of silver nanoparticles were screened against human Hep2 cell lines by means of MTT assay. Reduction of silver ions by the algal culture was observed during 72 h of incubation and the synthesized nanoparticles were further characterized.

Applications

Silver nanoparticles synthesized from S. platensis cold-tolerant strain can be used as potential antibacterial and anticancerous agent. Antibacterial and anticancerous silver nanoparticles synthesized from cold-resistance strain of S. platensis would suggests the possible utilization of biogenic silver nanoparticles as an effective and biocompatible biocide.

Peer review

In this research the authors studied the silver nanoparticles synthesized from S. platensis cold-tolerant strain against Gram-negative organism P. aeruginosa and Gram-positive organism S. aureus. Antibacterial and anticancerous silver nanoparticles synthesized from cold-resistance strain of S. platensis would suggests the possible utilization of biogenic silver nanoparticles as an effective and biocompatible biocide.

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