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Antibacterial screening of silver nanoparticles synthesized by marine micro algae

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ABSTRACT

Objective: To explore the biosynthesis of silver nanoparticles synthesized by marine microalgae. **Methods:** Marine microalgae was collected from Central Marine Fisheries Research Institute (CMFRI, tuticorin) and cultured in the lab. Silver nanoparticles synthesis were observed in normal and microwave irradiated microalgae and screened against human pathogens for the presence of antimicrobials. **Results:** The presence of silver nanoparticle was confirmed by UV-Visible spectroscopy at 420 nm by the presence of plasmon peak. Further confirmation was done by scanning electron microscope (SEM). **Conclusions:** These results not only provide a base for further research but are useful for drug development in the present and future.

1. Introduction

Nanotechnology provides the ability to engineer the properties of materials by controlling their size, and this has driven research toward a multitude of potential uses for Nanomaterials. Metallic nanoparticles exhibit unusual optical, thermal, chemical, and physical properties. The reduction of materials' dimension has pronounced effects on the physical properties that may be significantly different from the corresponding bulk material. Some of the physical properties exhibited by nanomaterials are due to large surface atom, large surface energy, spatial confinement, and reduced imperfections[1]. Nowadays, scientists try to achieve a wide range of possible applications of nanotechnology-enabled and environmentally friendly manufacturing processes that reduce waste products, ultimately leading to atomically precise molecular manufacturing with zero waste. The use of nanomaterials as catalysts for greater efficiency in current manufacturing processes by minimizing or eliminating the use of toxic materials, the use of nanomaterials and nanodevices to reduce pollution (e.g. water and air filters) and the use of nanomaterials for more efficient alternative energy production (e.g. solar and fuel cells) are some examples for the application of nanoparticles[2].

Unfortunately, there is a flip side to these benefits. As scientists experiment with the development of new chemical or physical methods to produce nanomaterials, the concern for a negative impact on the environment is also heightened. Some of the chemical procedures involved in the synthesis of nanomaterials use toxic solvents, could potentially generate hazardous byproducts, and often involve high energy consumption, and not to mention the unsolved issue of the potential toxicity of certain nanomaterials. This is leading to a growing awareness of the need to develop clean, nontoxic and environmentally friendly procedures for synthesis and assembly of nanoparticles. A lot of interest has been created by the term "green nanotechnology" with the flourishing demand of "green" nanoparticle synthesis processes[2]. The field of nanoparticle synthesis has recently developed new routes. Biosynthetic methods employing either biological microorganisms or plant extracts have emerged as a simple and viable alternative to chemical synthetic procedures and physical methods[3]. Although it is known that microorganisms such as bacteria, yeast and now fungi play an important role in remediation of toxic metals through reduction of the metal ions, this was considered interesting as nanofactories very recently.

It has been known for a long time that in nature a variety of nanomaterials are synthesized by biological processes. For example, the magneto tactic bacteria synthesize intracellular magnetite or greigite nanocrystallites, the other examples are diatomas, which synthesize siliceous materials and S-layer bacteria that produce gypsum and calcium

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carbonate layers. These results showed that microorganisms could indeed be used for the synthesis of nanoparticles.

Herein, a novel combinatorial synthesis approach which is rapid, simple and "green" for the synthesis of metallic nanostructures of noble metals such as silver (Ag), by using a combination of algal culture and microwave (MW) irradiation in water in absence of a surfactant or soft template are described. These works will help to encourage research by which more value added products can be obtained from the algae *Chaetoceros calcitrans (C. calcitrans)*, *Chlorella salina(C. salina)*, *Isochrysis galbana (I. galbana)* and *Tetraselmis gracilis (T. gracilis)*.

2. Materials and methods

2.1. Marine micro algae cultures

The algal species *C. calcitrans*, *C. salina*, *I. galbana* and *T. gracilis* were collected from Central Marine Fisheries and Research Institute (CMFRI) Tuticorin. The stock was maintained in Walne's media. The growth of each alga was studied by measuring their absorbance values at their respective λ max. The exponential phase of algal cells was observed for the first 15 days.

2.2. Synthesis of silver nanoparticles[1]

The synthesis of silver nanoparticles from marine micro algae were carried out by two methods namely normal marine microalgae and microwave irradiated marine microalgae. For this, the algal cultures from mid exponential phase of its growth were collected and further the experiments were carried out in six methods. For all the methods control experiments were conducted to check the role of algae in silver nanoparticle production.

2.2.1. Synthesis of silver nanoparticles by normal marine microalgae

There were three methods used for the synthesis of silver nanoparticles by normal marine microalgae. In the first method the algae was cultivated along with 1mM $AgNO_3$ solution and then kept in shaker incubator for two weeks. In the second method the algal cultures were procured at the mid of exponential phase and subjected to centrifugation at 5 000 rpm for 5 mins. Silver nitrate solutions were added to both pellet and supernatant. In the third method the cells were subjected to ultrasonication and then centrifuged. Again 1mM $AgNO_3$ solution was added and kept in shaker for two weeks.

2.2.2. Synthesis of silver nanoparticles by microwave irradiated marine microalgae

In this method, the algal culture were collected from mid exponential phase of its growth and subjected to microwave irradiation of 5 seconds and 15 seconds off for 5 times. Further experiments were carried as described above.

2.3. Antimicrobials sensitivity test

Antimicrobials sensitivity test was done to detect whether silver nanoparticle has any antagonistic character against pathogens like *Escherichia coli (E. coli)*, *Klebsiella* sp, *Proteus* sp and *Pseudomonas* sp by using Muller Hinton agar and incubated at 37 ℃ for 24 hrs.

3. Results

The stationary phase of algal cells was observed after 15 days of its growth and then started to decline (Figure 1).

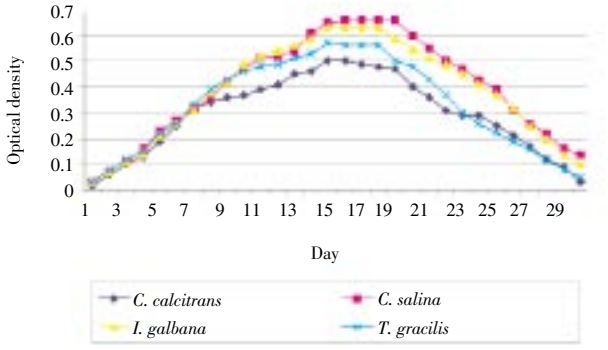

Figure 1. Growth curve of algae (*C. calcitrans*, *C. salina*, *I. galbana* and *T. Gracilis*).

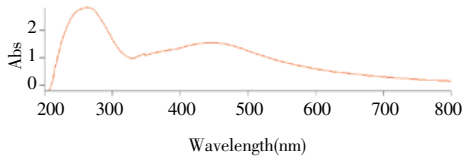

Figure 2. UV-Vis spectra recorded for the presence of aqueous $AgNO_3$ solution.

3.1. Synthesis of silver nanoparticles

The appearance of brownish black color in solution suggested the formation of silver nanoparticles with the plasmon resonance peak at 420 nm. Thus, it was evident that the metabolites excreted by the culture exposed to silver could reduce silver ions, clearly indicating that the reduction of the ions occur through electron shuttle or through reducing agents released into the solution by algal culture. These reactions only occurred in the light and the nanoparticles were not produced in the darkness. On the other hands, the reduction of silver ions did not occur in the absence of algal cells. The plasmon resonance observed at 420 nm for silver nanoparticles produced by normal and microwave irradiated marine microalgae shown in Figure 2. The color change in the algal culture due to silver nanoparticles produced is by scanning electron microscope (SEM) (Figure 3).

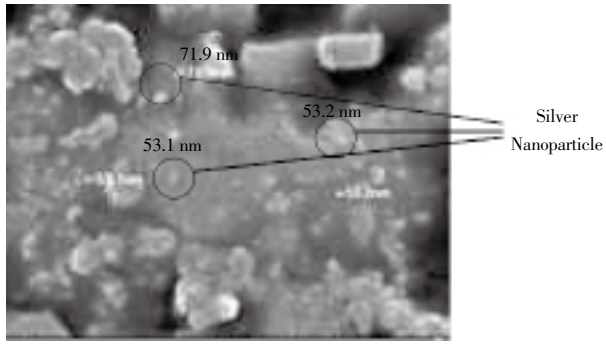

Figure 3. SEM image of the silver nanoparticle produced by marine microalgae.

3.2. Antimicrobial sensitivity test

The human pathogens *Klebsiella* sps, *Proteus vulgaricus*, *Pseudomonas aerujinosa* were checked with normal algae to find the presence of antimicrobial activity. The silver nanoparticles produced by *C. calcitrans* showed inhibition against *Klebsiella* sps, *Proteus vulgaris(P. vulgaris)*, *Pseudomonas aeruginosa(P. aeruginosa)* and against *E. coli*. The silver nanoparticle produced from *C. salina*, *I. galbana* and *T. gracilis* also showed better zone of inhibition against *Klebsiella* sps, *P. vulgaricus*, *P. aeruginosa* and against *E. coli* (Figure 4).

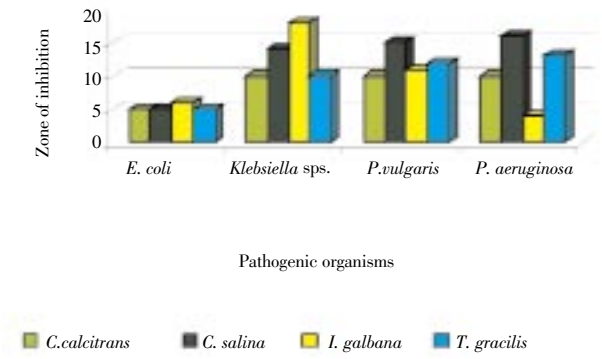

Figure 4. Antimicrobial activity of silver nanoparticles produced from microalgae against pathogens.

4. Discussion

The synthesis of nanoparticles is in the lime light in modern nanotechnology. The development of biologically inspired experimental processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. In the present study 4 microalgal strains were isolated and screened for the synthesis of silver nanoparticles. Upon addition of Ag^+ ions into the cell free culture in the dark, samples changed color from almost colorless to dark brown with intensity increasing during the period of incubation . It showed no change in color of the cell filtrate when incubated in the same condition. The appearance of a yellowish brown color in solution was a clear indication of the formation of silver nanoparticles in the reaction mixture. A characteristic surface plasmon absorption peak at 420 nm was observed at 24 hrs that attained the maximum intensity after 72 hours. The plasmon bands are broad with an absorption tail in the longer wavelengths, which could be in principle due to the size distribution of the particles[4]. The stability of the synthesized silver nanoparticles was studied by measuring its intensity at 420 nm over a period of one month in room temperature. No significant change in the intensity was observed which proved its stability over a period of one month while the peak at 234 nm maybe due to absorption by amide bond. This indicates secretion of some protein components into the medium by the fungal biomass which plays an important role in the reduction of the metal ions in the form of nanoparticles. Consequently, the proteins may also bind to the nanoparticles and enhance stability.

Metallic silver is relatively unreactive, however, when exposed to aqueous environments some ionic silver (Ag^+) is released. Certain salts (e.g. silver nitrate) are readily soluble in water and have been exploited as antiseptic agents for many decades[5]. Silver nanoparticles have been demonstrated to exhibit antimicrobial properties against bacteria[6] with close attachment of the nanoparticles themselves with the microbial cell and the activity being size dependent[7]. The size and structure of nanoparticles was further characterized using SEM analysis. The surface deposited silver nanoparticles are clearly seen at high magnification in the micrograph.

The rapid biological synthesis of silver nanoparticles by marine microalgae provides a simple and efficient route for the synthesis of nanoparticles with tunable optical properties directed by particle size. Investigation on the antibacterial effect of nanosized silver colloidal solution against human pathogens reveals high efficacy of silver nanoparticles as a strong antimicrobial agent which can be useful in food industries, cosmetic industries and in pharmaceuticals.

Future prospects of this research would be to scale-up the biosynthetic production of silver nanoparticles using these algae and to prove its efficacy against a wide spectrum of microbial population. Further investigations would involve exploring the potency of micro algae to synthesize gold nanoparticles.

Conflict of interest statement

We declare that we have no conflict of interest.

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