Egypt. J. Bot., **Vol**. **56,** No.2 pp. 371-379 (2016)

Biosynthesis of Silver Nanoparticles from the Marine Microalga *Isochrysis galbana* and their Antibacterial Activity Against Pathogenic Bacteria

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CURRENTLY, there is a growing need to develop environmentally benign nanoparticle synthesis process that does not use toxic chemicals in the synthesis protocols. Synthesis of nanomaterials by biological approach is innovative, cheaper and environmentally friendly. In this regard, the present study focused on the synthesis of silver nanoparticles from the microalga *Isochrysis galbana.* The silver nanoparticle produced by marine microalga *Isochrysis galbana* when incubated with silver nitrate solution at the same culture condition for 24 h and was detected by UV-Vis spectrophotometer, Energy dispersive X-ray (EDX-ray) and Transmission electron microscope. The synthesized silver nitrate nanoparticles from the marine microalga *Isochrysis galbana* showed the pronounced antibacterial activity against the tested human pathogens *Escherichia coli* and *Proteus vulgaris*. The above eco-friendly synthesis procedure of silver nitrate nanoparticles could be easily scaled up in future for the industrial and therapeutic needs.

Keywords Silver nanoparticles, *Isochrysis galbana,* Antibacterial activity.

An important aspect of nanotechnology is the development and synthesis of nanoparticles which is considered as a big challenge. Nanoparticles are being viewed as fundamental building blocks of nanotechnology. The development of the biologically inspired experimental process for the synthesis of nanoparticles is evolving into an important branch of nanotechnology (Dhanalakshmi *et al*., 2012). Nanoparticles of metals have been extensively researched because of their unique physical properties, chemical reactivity and potential applications in catalysis, biological labeling, biosensing drug delivery, antibacterial activity, antiviral activity, detection of genetic disorders, gene therapy and DNA sequencing (Thirumurugan *et al*., 2010).

Benthic marine algae are the group of algae that live either in marine or brackish water environment. The synthesis of nanoparticles using algae as a source has been unexplored and underexploited. More recently, there are few

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investigations reported that algae being used as a biofactory for synthesis of metallic nanoparticles. In an important report, Singaravelu *et al*. (2007) implemented an efficient approach for the synthesis of stable gold nanoparticles by the reduction of aqueous $Aucl_4$ by using *Sargassum wightii*. Recently Kumar *et al*. (2012) showed higher antibacterial activity of silver nanoparticles synthesized by *Sargassum tenterrimum* compared to the phytochemicals present. Devina Merin *et al*. (2010) and Kathiraven *et al*. (2014) showed that the marine microalgae rapidly synthesize silver nanoparticles with high efficient of antibacterial activity against human pathogens.

The silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine. A number of living organisms are already well known to elaborate silver nanostructured compounds such as cyanobacteria, bacteria , actinomycetes and plants as *Cinnamomum camphora* (Huth and Kwon, 2011) and *Pelargonium graveolens* (Lukman *et al*., 2011). Biosynthesis of silver nanoparticles using the marine seaweed *Sargassum wightii* was carried out by Shanmugam *et al*. (2013). In our present study, we report the synthesis of silver nanoparticles using the marine microalga *Isochrysis galbana* and also assess their antagonistic effect against some pathogenic bacteria.

Material and Methods

Tested alga

The marine microalga *Isochrysis galbana* (Haptophyceae) was obtained from the Institute of Oceanography and Fisheries in Alexandria (ARE) The algal culture was maintained in Walne's medium under uEs -1m -2 light intensity with photoperiod of 16/8 light / dark cycle at 25°C . The growth of *Isochrysis galbana* was monitored by measuring the optical density (OD) at 600nm (with a Pharmacia Biotech spectrophotometer). The exponential phase of algal cells was recorded for the first 15 days.

Synthesis of silver nanoparticles and preparation of extract

The biosynthesis of silver nanoparticles from marine microalga *Isochrysis galbana* was performed as follow; the algal culture from mid exponential phase of its growth at ninth day was collected by centrifugation at 5000 rpm for 5 min and the pellets were washed with sterile distilled water to remove the traces of media salts. The cell filtrate and biomass of 5ml was mixed with 95 ml of silver nitrate solution (1mM) and incubated in the previous temperature and light conditions for 24 hr. During the incubation period, change in color of culture from pale yellow to dark brown indicates nanoparticle synthesis. Capping of micro algal proteins metabolites and reduction of silver ions may lead to formation of silver nanoparticles in the solution (Sudha *et al*., 2013).

Antibacterial sensitivity test

The microorganisms used for the antibacterial activity assay were *Escherichia coli* (E. coli) and *Proteus vulgaris* (gram negative bacteria) which were obtained from National Institute of Oceanography and fisheries, Alexandria. The antibacterial sensitivity test was done to detect whether silver

nanoparticle has any antagonistic effect against the chosen pathogenic bacteria using Muller Hinton agar and blood agar plates and gel puncture. The pH of medium was maintained at 7.4 and then it was sterilized by autoclaving at 121°C and 15 Ibs pressure for 15 min. 20 ml of the sterilized medium was poured into sterilized petri dishes and allowed to solidify at room temperature. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. In each of these plates 5-mm diameter wells were made at the center using an appropriate size sterilized cork borer. Different concentrations of the nanoparticles solution (5, 10 and 20 µl) were poured into each well (5-mm) on all plates (Nathan *et al*., 1978). After incubated at 37°C for 24 hr, the different zones of inhibition of bacteria were measured (mm) (Senthil and Kamaraj, 2011). The assays were performed in triplicate.

Detection and confirmation of silver nanoparticles

Ultraviolet (UV-vis) spectrum (Shimadzu UV- 1601 spectrophotometer), EDX ray analysis as well as transmission electron microscopy were used to detected and confirm the nanoparticle production.

Results

The stationary phase of *Isochrysis galbana* algal cells was recorded after 15 days of culturing and then growth started to decline (Fig. 1).

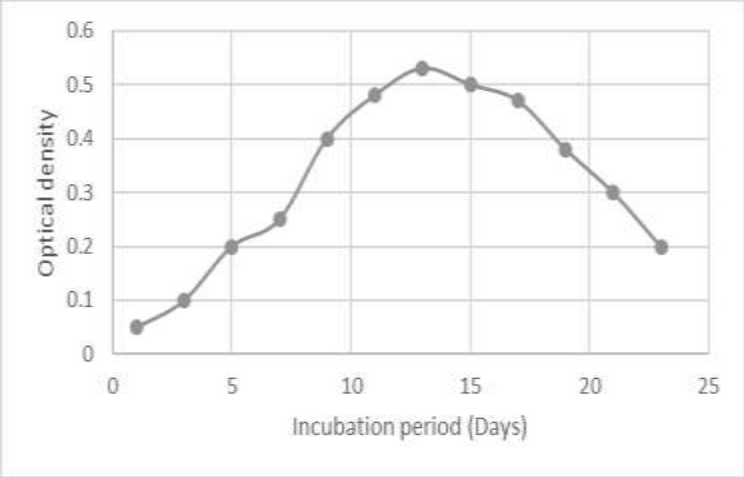

Fig. 1. Growth curve of *Isochrysis galbana*, each value is a mean of five replicates.

Synthesis of silver nanoparticles

The appearance of brownish black color in the culture solution of *Isochrysis galbana* suggested the formation of silver nanoparticles with the plasmon resonance peak at 440 nm. Thus, it was evident that the metabolites excreted by the alga exposed to silver could reduce silver ions, this may indicate that the reduction of the ions occur through electron shuttle or through reducing agents released into the solution by the alga. These reactions only occurred in the light and the nanoparticles were not produced in the dark. On the other hands, the reduction of silver ions did not occur in the absence of algal cells. The plasmon resonance observed at 440 nm for silver nanoparticles produced by normal marine microalga was shown in Fig. 2. The transmission electron microscopy has been used to identify the silver nanoparticles produced by marine microalga *Isochrysis galbana* (Fig. 3). Energy-dispersive X-ray (EDX) spectroscopy analysis for the confirmation of elemental silver was carried out for the detection of bioavailable metals at the subcellular level of an organism which may be bounded to a suspended particle of tested organism. (Fig. 4).

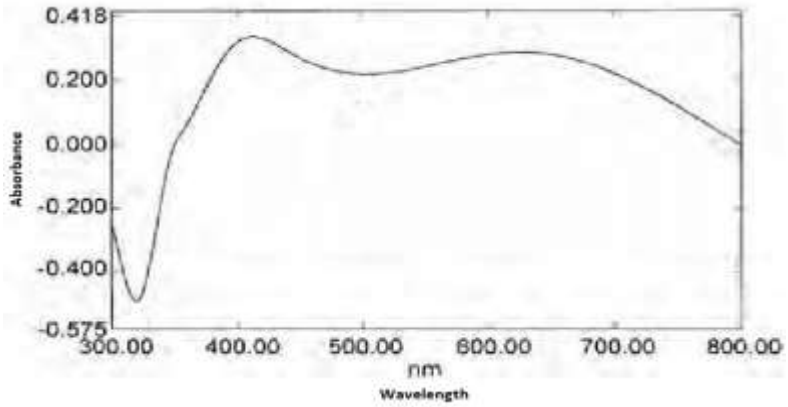

Fig. 2 . The UV- Vis spectra of silver nanoparticles produced. (Silver nitrate solutions incubated with marine microalga *Isochrysis galbana*).

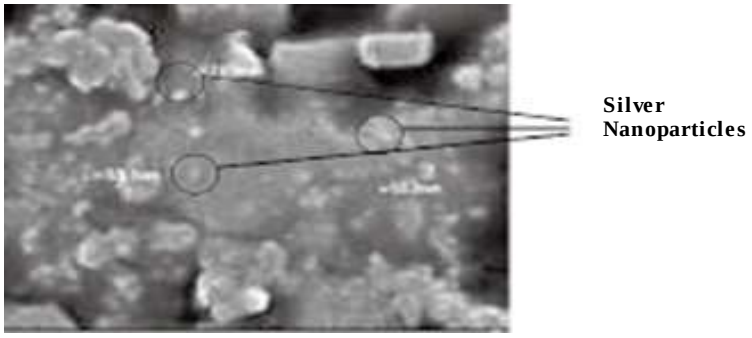

Fig. 3. Transmission electron microscope of the silver nanoparticles produced by marine microalga *Isochrysis galbana*

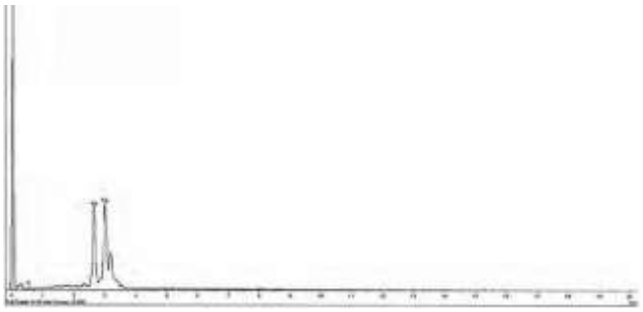

Fig. 4. Energy-dispersive X-ray (EDX) analysis of the silver nanoparticles produced by marine microalga *Isochrysis galbana* confirmed the elemental composition of nanoparticles as silver

Antibacterial sensitivity test

The results obtained from the present study concerning the biological activity of the antibacterial agents produced by *Isochrysis galbana* synthesized silver nanoparticles against two different species of bacteria with various concentrations (5, 10 and 20µl) were recorded in Table 1 and Fig. 5. The maximum antibacterial activity was found in *Proteus vulgaris* (17mm for 20 µl) and minimum level of antibacterial activity was noticed in *Escherichia coli* (6 mm for 5 µl). Antibacterial activity was shown to be concentration dependent, *i.e* the diameter of inhibition zone increased with elevation of solution concentration (5, 10 and 20 µl).

TABLE 1. Antibacterial activity of sliver nanoparticles against *Escherichia coli* and *Proteus vulgaris* (each value is a mean of three replicates).

Test bacteria	**Concentration of silver nanoparticle solution (µl)**	5	10	20
Escherichia coli	Zone of inhibition (mm)	6	9	14
Proteus vulgaris		8	11	17

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, *Isochrysis galbana* was collected from the mid exponential phase of its growth and was cultivated along with silver nitrate solution. Upon addition of Ag^+ ions into the cell free culture in the light, samples changed color from pale yellow to dark brown with intensity increasing during

the period of incubation. It showed no change in color of the cell filtrate (free algal cells) in control when incubated in the same condition. The appearance of a yellowish to dark brown color in solution was a clear indication of the formation of silver nanoparticles in the reaction mixture. The mechanism of action of silver nanoparticles synthesis was not known, but it was hypothesized that the silver ions required the NADH-dependent nitrate reductase enzyme for their reduction (Labrenz *et al*., 2000 and Shankar *et al*. (2003). Shankar *et al*., 2003 suggested that shoulder at 370 nm (UV-vis spectra in Fig. 2) corresponded to the transverse plasmon vibration in silver nanoparticles, whereas the peak at 440 nm was due to excitation of longitudinal plasmon vibration (Shankar *et al*., 2004). After 24 hr, the process was stopped and the particles were further detected by transmission electron microscope. The energy dispersive X-ray spectroscopy analysis of the silver nanoparticles confirmed the elemental composition of nanoparticles as silver by sharp signals (Fig. 4). The optical absorption band peak in the range of 3-4 KeV is typical for the absorption of metallic silver nanocrystallites recorded by Magudapathy *et al*. (2001).

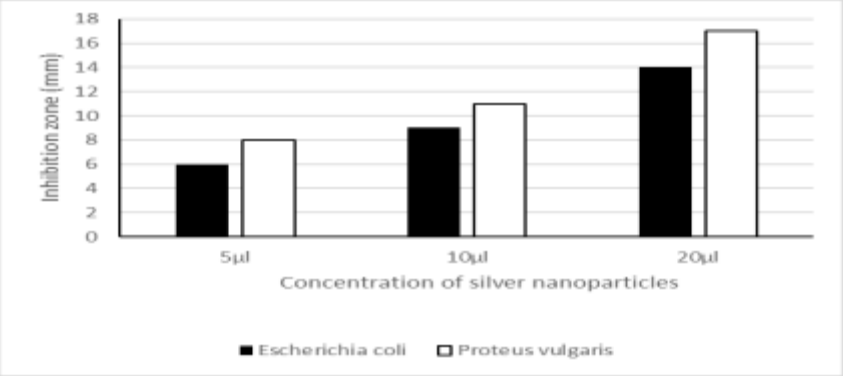

Fig. 5. Antibacterial activity of sliver nanoparticles against *Escherichia coli* and *Proteus vulgaris*.

The antibacterial activity of *Isochrysis galbana* synthesized silver nanoparticles were tested against *Escherichia coli* and *Proteus vulgaris* using various concentrations (5, 10 and 20 µl) and results were shown in Table 1 and Fig. 5. Maximum zone of inhibition was found in *Proteus vulgaris* (17mm for 20 µl) and the minimum antibacterial activity was observed in *Escherichia coli* (6mm for 5 µl) . This difference in activity may be due to the susceptibility of the organism used in the current study. The nanoparticles are attached to the cell membrane and penetrated inside the bacterial cells. When silver nanoparticles enter the bacterial cell, it forms a low molecular weight compound in the center of the bacteria to which the bacteria conglomerates thus protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain cell division finally leading to the cell death (Kvitek *et al*., 2008). The nanoparticles release silver ions in the bacterial cells which enhance their

bacterial activity (Morones *et al*., 2005 and Kathiraven *et al*., 2014). Several studies proposed that silver nanoparticles may be attached to the surface of the cell membrane, disturbing permeability and respiration functions of the cell (Morones *et al*., 2005 and Rajeshkumar1 *et al*., 2013). It may also possible that silver nanoparticles not only interact with the surface of membrane, but it can also penetrate inside the bacteria (Sondi and Salopek, 2007).

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 exploited as antiseptic agents for many decades (Lansdown, 2002). Silver nanoparticles have been demonstrated to exhibit antibacterial properties against certain pathogenic bacteria (Sondi and Salopek, 2007).

Conclusions

It is concluded that 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 revealed high efficacy of silver nanoparticles as a strong antibacterial agent, which can be useful in food industries, cosmetic industries and in pharmaceuticals. In addition, they are eco-friendly, economically low cost, … ect. Future prospects of this research would be to scale-up the biosynthetic production of silver nanoparticles using this alga and to prove its efficacy against a wide spectrum of microbial population.

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(*Receved* 12/8/2015;
*accepted*16/9/2015)

انتاج جسيمات الفضة متناهية الصغر من الطحلب البحري ايزوكريسيس جلبانا واستخدامها كمضاد لنشاط البكتريا المسببة للأمراض

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اتجه العالم الى انتاج مركبات حيوية من الطحالب بديلا عن المركبات ذات الاثار الجانبية السامة٠

ركزت الدراسة على انتاج جسيمات الفضة متناهية الصغر من طحلب البحري ايزوكريسيس جلبانا وذلك بان تم تحصين خلايا الطحلب(وهي في منتصف مرحلة النمو) مع محلول نترات الفضة (١ مليمول) في نفس ظروف النمو من شدة الإضاءة والتواقيت الضوئي ودرجة الحرارة ولمدة ٢٤ ساعة٠

اثناء فترة التحصين يتغير لون الوسط من الأصفر الى البني الداكن دليل على تكوين جسيمات الفضة متناهية الصغر وللتحقق من تكوينها تم استخدام الاشعة البنفسجية والسينية والمجهر الالكتروني الناقد٠

واظهرت النتائج ان لهذه الجسيمات نشاط مضاد لبعض أنواع البكتريا الممرضة مما يشجع على استخدامها مستقبلا على نطاق واسع في مجال صناعة وإنتاج الدواء٠