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Characterization and Applications of the Biosynthesized Silver Nanoparticles by Marine *Pseudomonas* sp. H64

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Nanoparticles have become important scopes of research. The present study made insight into the using of marine bacteria as a new ecofriendly and low cost source for biosynthesis of silver nanoparticles. Fifteen marine bacteria were tested for their capabilities to synthesize silver nanoparticles. Characterization of silver nanoparticles synthesized by the most promising *Pseudomonas* sp. H64 was performed using UV–Visible spectrophotometer, Fourier transform-infrared spectroscopy, zeta potential, transmission electron microscopy and X-ray. The produced Ag NPs are spherical particles with size of 3-22 nm and negative charge (-14 mV). Different valuable applications of the biosynthesized AgNPs were investigated. The produced AgNPs exhibited promising antibacterial activity against human and fish pathogens (*Vibrio parahaemolyticus*, *Aeromonas hydrophila*, *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis*) with inhibition zone diameters (10-37 mm). Moreover, antifungal activity against (*Fusarium solani*, *Rhizoctonia solani; Rhizopus oryzae*, *Helminthosporium* sp.) expressed as inhibition of growth was (77.8±0.68-100%), while no antifungal activity was detected against *Aspergillus niger*. Antifouling and antioxidant activities were also confirmed. AgNPs exhibited anticancer activity toward (HepG-2, MCF-7, CaCo-2) cell lines. Moreover, AgNPs exhibited bioremediation potential of heavy metals and dyes.

Keywords: Silver nanoparticles; antibacterial; *Pseudomonas* sp. H64.

Nanobiotechnology is a miscellany of biotechnology and nanoscience. Nanoparticles with sizes below 100 nm are applicable in different fields of biomedicine [1]. Traditional methods for production of nanoparticles cause harmful effect to the environment. This leads to a growing interest for the development of non toxic and environmentally friendly sources for production of nanoparticles[2]. Metal nanoparticles can be produced using natural marine resources as alternatives to the physical and chemical methods including plants, fungi, bacteria and algae [3].

Metal ions can be grabbed by the microorganisms from their environment and then turn into the element metal through reduction/ oxidation mechanism using some enzymes with reducing or antioxidant properties which act on the compounds and result in the production of nanoparticles [4].

Researchers and pharmaceutical companies are searching for new antimicrobial agents due to arising of antibiotic resistant pathogenic strains causing infectious diseases[5,6]. Silver nanoparticles are firmly the best used nanomaterials as antimicrobial agents. Besides possessing antibacterial property, silver nanoparticles (AgNPs) seem to be good conductor, catalysts, antifungal agents, antiviral agents, anticancer, anti-inflammatory and antifouling [7-11]. In addition, nano-bioremediation technology has been developed to facilitate the bioremediation

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of organic pollutants including synthetic dyes and water treatment[12].

According to our knowledge, few literatures concerned with the green synthesis of silver nanoparticles by marine bacteria. Thus, in the current study an attempt has been made towards synthesis of silver nanoparticles by marine bacteria as a new and ecofriendly marine source. Further characterization and evaluation of the antimicrobial, antifouling, anticancer and antioxidant activities in addition to removal of dye and heavy metals will be investigated.

MATERIALS AND METHODS

Microorganisms

The marine bacteria were provided by Dr. Gehan Abou Elela. National Institute of Oceanography and Fisheries, Alexandria, Egypt.

Pathogenic indicators

Different pathogenic indicators of Gram-negative bacteria (*Vibrio parahaemolyticus*, *Aeromonas hydrophila*, *Escherichia coli*), Gram-positive bacteria (*Streptococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis*) and fungi (*Fusarium solani*, *Rhizoctonia solani, Rhizopus* oryzae, *Helminthosporium* sp. and *Aspergillus niger*) were kindly provided by Marine Microbiology Department, Environment division, National Institute of Oceanography and Fisheries, Alexandria, Egypt

Growth condition and AgNPs biosynthesis

Pure culture of *Pseudomonas* sp. H64 was cultured on nutrient broth medium of the following components (g/l): beef extract, 1; yeast extract, 3; 1.5; peptone, 5 at pH 7 to synthesize the AgNPs[13].The bacterial culture was incubated for 24 h under shake condition at 150 rpm and 30 ^{o}C. The supernatant of the overnight bacterial culture was collected after centrifugation at 5000 g for 10 min. and retained for AgNPs biosynthesis. Fifty millilitre of cell-free supernatant was added to individual reaction vessels each containing fifty milliliter of $AgNO_3$ solution (1 mM final concentration). The reaction mixture was incubated at 30 ^{o}C in the dark for 24 h. Visual observation of change in colour of the reaction mixture was regarded as positive indication of AgNPs biosynthesis compared with control (Absence of Ag NO_3). The formation of nanoparticle was examined under UV-visible spectrophotometer at 24h time interval [6].

Characterization of AgNPs

UV–Visible spectrometric analysis

Confirmation of AgNPs production was performed using UV–vis spectral analysis using UV–Vis spectrophotometer (Helios alpha, Unican) at wavelength of 300–800 nm [14].

Fourier- Transform Infrared (FTIR) spectroscopy analysis

FTIR spectroscopy was carried out to determine the functional groups on the biosynthesized silver nanoparticles[15], which are responsible for stability of the nanoparticle. The purified dried silver nanoparticles were mixed with potassium bromide in the ratio of 1:100 then analyzed in a FTIR (Perkin–Elmer Spectrum RX1, Shelton, Connecticut) with spectrum (450–4000 cm^{-1}) and resolution of 4 cm^{-1} .

Zeta potential

Zetasizer (ZS 90, Malvern, UK) was used for analysis of surface charge on AgNps. Samples were exposed to dilution using 0.15M phosphate buffer saline at pH 7.2, then were examined in dynamic light scattering cuvettes by zeta potential within the range of 0.1–10000 nm at a scattering angle of 90° and 25°. This was done at Faculty of Pharmacy, Alexandria University.

Transmission electron microscope (TEM)

The morphology and size of AgNPS were characterized by TEM (TECNAI SPIRIT). It was carried out at the National Institute of Oceanography and Fisheries.

Energy dispersive X-ray analysis (EDX)

EDX was achieved by X-ray microanalysis system joined with SEM to confirm the presence of AgNPs and to observe the elementary compositions of the particles [14]. It was investigated at the Faculty of Science, Alexandria University.

Preparation of Ag NPs stock solution

AgNPs (5 mg/mL) stock solution was prepared using sterilized deionized water. It was saved at 4^{o}C and used within seven days. Sonication of the stock solution for 10 min and vortex for 1 min was done before use.

Antibacterial activity

Disk diffusion test [16] was applied to estimate the antibacterial effect of AgNPs. Briefly, the tested bacterial cultures were grown for overnight in nutrient broth, then separately lawn cultured on the prepared nutrient agar plates.

Sterile filter paper discs (5 mm) were saturated by AgNPs solution and placed above the culture and incubated at 37°C for 24h. The diameter of inhibition zone was measured in millimeters.

Antifungal activity

Antifungal activity of AgNPs against *F. solani*, *R. solani; R. oryzae*, *Helminthosporium* sp. and *A. niger* was detected by assay of growth inhibition of the tested fungi[17] with some modifications. 50 µl of AgNPs solution was added to potato dextrose medium (PDA) to obtain the required concentration. A disc of each indicator fungus (8mm diameter) was put on the presterilised PDA medium and incubated at 30 °C. AgNPs free medium was taken as control. Colony diameter was measured after 72 h and compared with the control (without addition of AgNPS). Inhibition % of the fungal growth was estimated using the formula: I = C – T/ C Õ 100 Where I = percentage inhibition; C = radial growth in control, T = radial growth in treatment (Test).

Antifouling activity

Antifouling activity

Antifouling activity was performed according to Abd-Elnaby et al. (2016)[11] with some modifications. Briefly, 200 µl of AgNPs was added to 1ml of Seawater in conical flask (50 ml) containing cover glass and incubated at 28°C for 24h. Then the cover glass was exposed to dying with crystal violet solution (0.4%) for ten minutes, followed by washing by water, dried at room temperature and observed under the microscope . A control flask with out AgNPs was taken as control.

Antioxidant activity

In vitro free radical scavenging activity of the biosynthesized AgNPs, was estimated with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Reduced AgNPs were dissolved in 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100mg/mL methanol; an equal amount of methanol was added to the control. DPPH solution (2 mL) in ethanol (100 mmol/l) was individually mixed with 2 mL of each concentration of methanol [13] followed by dark incubation (15 min); thereafter, the optical density was indicated at 517 nm against the blank, and ascorbic acid was taken as control. The scavenging percentages of DPPH with the $AgNP_s$ were calculated using the following formula: S_{fr} (%) =(A_c -As/A_c) x100%, where S_{fr} is free radical scavenging, Ac is the absorbance of the control (ascorbic acid) and As is the absorbance obtained from the sample.

Anticancer activity

The anticancer activity of the AgNPs at concentration (0.39-25µg/ml) was tested against Intestinal carcinoma cells (CaCo-2); Hepatocellular carcinoma cells (HepG-2) and Breast carcinoma cells (MCF-7). Cell growth and survival was detected using MTT assay[18]. Proliferation reduction of cancer cells was estimated as follows:

Inhibition rate (%) = (1- Abs sample / Abs control) × 100

Bioremediation potential of Ag NPs (*Dye decolorization using AgNPs)*

Decolorization of four dyes namely Acid orange, Direct green, Acid black and Acid fast brown using the biosynthesized Ag NPs was investigated. Briefly, 1ppm concentration of each dye were prepared. Five test tubes, each containing 1ml of the prepared dye solutions were prepared and 0.1 mg of AgNPs were added to each tube, while one was maintained as blank. The test tubes were incubated at 30 °C for 2 h, then samples were centrifuged and the supernatant was used to measure the concentration of dye using UV-visible spectrophotometer according to the $ë_{max}$ of each dye[15]. Decolorization ratio expressed as mean value was estimated according to the following equation:

$$\text{Decolorization\%} = \frac{(C_o - Ce)}{C_o} \times 100$$

C_o= Initial absorbance before decolorization, Ce: final absorbance after decolorization

Bioaccumulation of heavy metal solutions

The uptake capacity of AgNPs to different heavy metals such as copper sulphate, nickel chloride, cadmium chloride and lead chloride with concentration of 100 ppm was investigated using batch adsorption experiment [19].

Bioremediation study

The biosynthesized AgNPs (0.1 mg) were added to 50 ml of metal solution and kept under shake condition at 120 rpm and 30 R"C for 24 h. The solution was centrifuged at 6900 g for separation of the absorbent. In the end of each experiment, the residual metal concentration in each solution was determined using an Atomic Absorption Spectrophotometer (Shimadzu-A-A—6800). Accumulation of metal ions was

evaluated using the following equation: q=V(IC-FC)/W, where q, metal accumulation; V, volume of reactions; IC, initial concentration (mg/l); FC, final concentration (mg/l) and W, total biomass [19].

RESULTS AND DISCUSSION

Bacterial mediated synthesis of silver nanoparticles

Fifteen bacterial species were tested for their ability to synthesize silver nanoparticles. Changes of the filtrate from yellow colour to yellowish-brown after addition of silver nitrate was used as indication of AgNPs formation[14]. The most promising isolate was *Pseudomonas*. sp. H64. It showed positive result in formation of AgNPs (Fig.1). Development of surface plasmon resonance in the mixture of the reaction is the main cause of change in the colour as was previously reported [20].

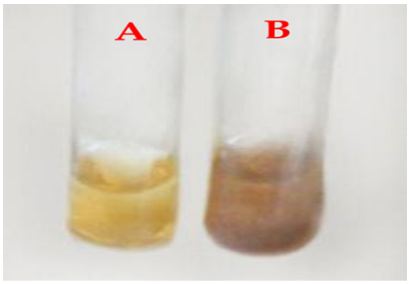

Fig. 1. Cell filtrate of *Pseudomonas* sp. H64 (before addition of $AgNO_3$) (A); treated with 1mM $AgNO_3$ (B)

1634.60 cm^{-1} which is characteristic to protein and 606.4 cm^{-1} which indicates the presence of C-l stretching of halides. Thus the present study confirmed the presence of amines, halides and proteins, which have strong binding affinity with Ag and help in capping, reducing and conversion of Ag^{+}ions to AgNPs. Similar results were previously reported [22,23]. It was stated that fabrication and stabilization of metal nanoparticles is dependent on different molecules including proteins, amines, phenolics, carbonyl groups, terpenoids [24]. Another study showed that proteins exhibit paired function of Ag+ reduction and shape-control during the synthesis of the silver nanoparticles[14].

Characterization of the biosynthesized AgNPs

AgNPs synthesis by *Pseudomonas*. sp. H64 was first confirmed by UV analysis. The absorption obtained indicated a characteristic peak for silver nanoparticles at 450 nm (Fig. 2). As it was reported, reduction of the silver ions to silver metal occurred in nanometer range by the action of extracellular reductase enzymes excreted by the microorganisms [14]. Some studies showed that the plasmon absorbance of AgNPs caused colour change of the reaction mixture to brown and the obtained peak was at 420 nm [21]. Screening for the functional groups included in the biosynthesized AgNps was accomplished using FT-IR analysis aiming at understanding the transformation process of silver nitrate into elemental silver. FT-IR spectrum at array of absorbance bands from 400-4000 cm-[1] (Fig. 3) showed three major peaks. Intense absorption bands at 3305. 98, which is characteristic to amine group(N–H stretch),

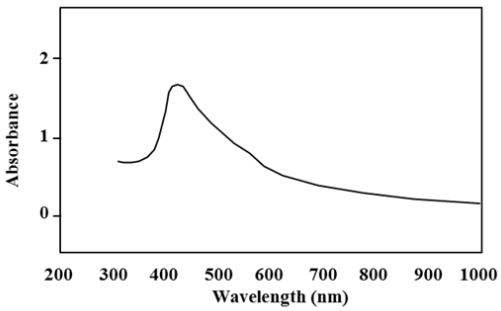

Fig. 2.UV-Visible absorption spectrum of silver nanoparticles synthesized by *Pseudomonas* sp. H64

Zeta potential

Zeta potential (f) is used to predict the interaction between particles in suspension. In the present study, the zeta potential value of *Pseudomonas. sp.* H64 mediated AgNPs was -14.4. mV (Fig. 4). Previous study reported that spherical Ag NPs showed potential of -5.1 mV and -15.3 mV[22]. Another study mentioned that charge of the AgNPs was -15.8 mV[17]. Disruption of charges on the surface plays a principal role in the assembly of the nanoparticles. Therefore, nanoparticles with reasonable zeta potential value may be applied as a drug providing a new horizon in avoiding resistance of bacteria and fungi [25].

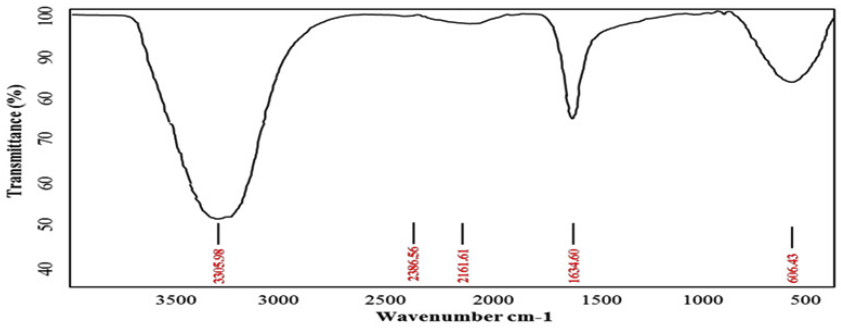

Fig. 3. FT-IR analysis of silver nanoparticles synthesized by *Pseudomonas* sp. H64

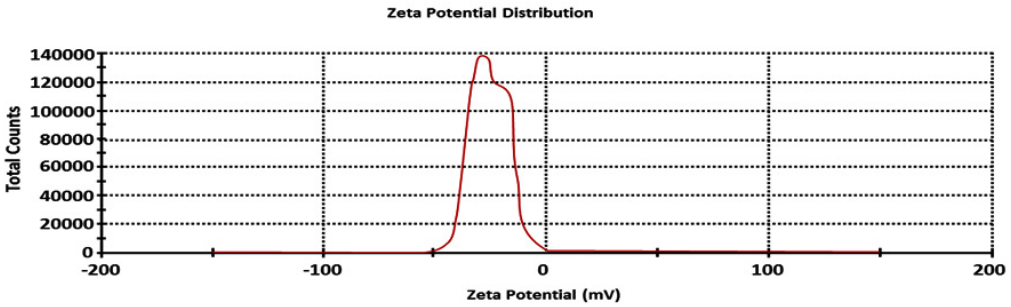

Fig. 4. Zeta potential of silver nanoparticles synthesized by *Pseudomonas* sp. H64

TEM

TEM micrographs of the purified nanoparticles (Fig. 5) showed the presence of silver nanoparticles with spherical shape, and size from 3-22 nm. It was reported that *Streptomyces* ERI-3 mediated synthesis of silver nanoparticles were relatively spherical with size 110-100 nm [26].It can be concluded that, the size ranges of silver nanoparticles synthesized by the marine of *Pseudomonas. sp.* H64 in the current investigation seemed closer to the size of AgNPs synthesized by other bacteria, which enable their use in different applications[27,28].

EDX

EDX analysis was done to study the X-ray analysis of silver peak in AgNPs. Results in Fig. 6 indicated that the absorption peak of AgNPs was observed at 3.5 keV, which represented a typical peak of metallic silver nanoparticles and confirmed that the main composition of the nanoparticles was silver as was reported [29,30]. Appearance of other peaks such as copper, sulphur, phosphorus and zinc, may be produced from the chemical and biological molecules like proteins and enzymes bound to the surface of the AgNPs[14,31].

Biotechnological applications of the biosynthesized AgNPs

Antibacterial activity

The biosynthesized AgNPs exhibited varied antibacterial activity against the tested pathogens (*B. subtilis, S. faecalis*, *S. aureus*, *E. coli*, *A. hydrophil, V. parahaemolyticus*) using disc diffusion method. As shown in Table 1, the highest antibacterial zone of inhibition (37±3.08 mm) was recorded against *A. hydrophila* (Fig. 7) followed by *E.coli* (32±2.67 mm). Abd-Elnaby et al.[19] demonstrated the antibacterial effect of the AgNPS synthesized by marine *Streptomyces rochei* MHM13 against the test pathogens including *Staphylococcus aureus* 25923, *Bacillus subtilis* 6633, *Escherichia coli* 19404, *Pseudomonas aeruginosa* 9027, *Bacillus cereus*, *Salmonella typhimurium* 14028, *Vibrio damsela* and *Vibrio fluvialis* with inhibition zone diameter (16-19 mm). Antibacterial activity of Ag NPs was proven in other studies [32-35]. Higher effect against Gram

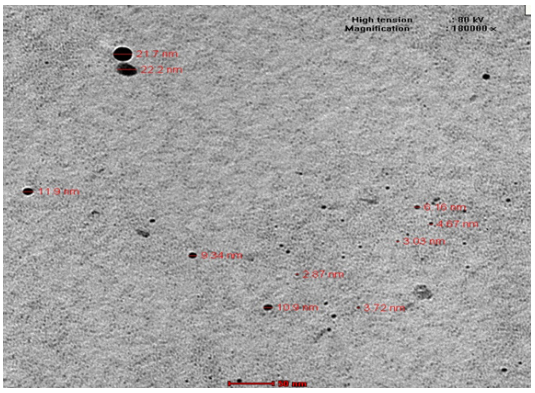

Fig. 5. TEM of silver nanoparticles synthesized by *Pseudomonas* sp. H64

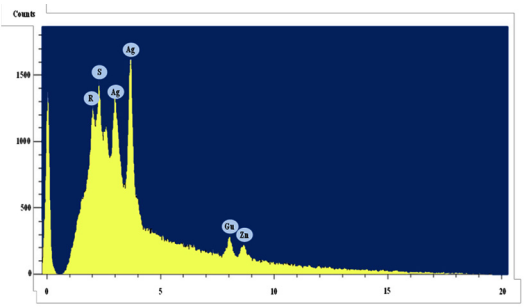

Fig. 6. X-ray microanalysis of silver nanoparticles synthesized by *Pseudomonas* sp. H64

Table 1. Antibacterial activity of AgNPs (expressed as inhibition zone diameter (mm) against different Gram positive and Gram negative bacterial pathogens

Bacterial pathogen	*S.aureus*	*S. faecali*	*B. subtilis*	*A.hydrophila*	*V. parahaemolyticus*	*E. coli*
Inhibition zone diameter (mm)	25±1.92	21±1.5	17±1.21	37±3.08	10±0.77	32±2.67

Table 2. Antifungal activity of Ag NPs (expressed as inhibition % of fungal growth)

Fungal pathogen	*A.niger*	*R.oryzae*	*R.solani*	*Helminthosporium sp.*	*F.solani*
Growth inhibition (%)	0	100±0.70	89±0.70	77.8±0.68	89±0.36

negative bacteria may be due to the existence of thin peptidoglycan layer compared to rigid peptidoglycan layer in Gram positive bacteria[36]. Moreover, the charged AgNPs exhibit better ability to bind the negative charges on cell wall of the Gram negative bacteria. The effect may also be attributed to binding of nanoparticles to bacterial membrane and release of hydrogen peroxide (H_2O_2) and super-oxide, which may lead to bacterial killing[37].

Antifungal activity

The present study extended to investigate the potency of the biosynthesized Ag NPs by *Pseudomonas.* sp. H64 as antifungal agents against *R. solani*, *R. oryzae F. solani, Helminthosporium* sp. and *A. niger.* Results shown in Table 2 revealed complete inhibition of *R.oryzae*. On the other side, silver nanoparticles caused 89%±0.36 and 89±0.70 % inhibition of *F.solani* and *R. solani,* respectively, while the lowest antifungal activity (77.8%±0.68) was against *Helminthosporium* sp. Absence of antifungal activity was observed against *A. niger*. Ali et al.[38] tested the antifungal activity of the biosynthesized AgNPs against *Fusarium graminearum*, *Fusarium solani* and *Penicillium expansum* and showed the inhibition of all tested fungi with the highest inhibition (91%) against *F. solani*. Efficiency of biosynthesized nanoparticles as fungicides was previously reported in different studies[38,39]. It was reported that the efficiency of AgNPs as antifungal agent is due to the presence of some components in the extract, which increase the reduction of the metals and stability of the resultant NPS[8].

Antifouling activity

Antifouling activity of the biosynthesized AgNPs was tested. Results (Fig. 8A) showed the inhibitory activity of AgNPs on biofilm formation compared to the control (Fig. 8B). This in agreement with that reported by Abd-Elnaby et al. (2016)[11], who confirmed the potential use of silver nanoparticles synthesized by marine *Strptomyces. rochei* HMM13 as antifouling agent.

Antioxidant activity

Natural antioxidants exhibit protecting effect of cells from oxidative damage[40]. The antioxidant activity of the AgNPs was detected using DPPH radical scavenging assay. The AgNPs achieved antioxidant activity with 84% inhibition at 100µg/ml. In parallel study, the antioxidant activity of AgNPs produced by *Bacillus aerius* caused 66.23% inhibition at 100µg/ml[30].

Anticancer activity

Anticancer activity of the Ag NPs (1.562-25µg/ml) was first detected by testing the toxicity against three cell lines: Intestinal carcinoma cells (CaCo-2); Hepatocellular carcinoma cells (HepG-2) and Breast carcinoma cells (MCF-7). Results in Fig. 9 showed that the viability of CaCo cells was (11.95-67.93 %), it was (12.40-90-24%) for HepG2 while the range of viability of MCF-7 was (14.24-32%) which means that the highest inhibitory activity was against MCF-7 and HepG2. The anticancer activity against the tested cell lines is shown in Fig. 10. The inhibition in the viability of the tested cell lines was dose dependent, which is in consistent with Abd -Elnaby et al.[19] who reported that the highest anticancer efficiency of the biosynthesized AgNPs was against HCT-116, MCF-7, Hep-G2 and A-549 cell lines, while the

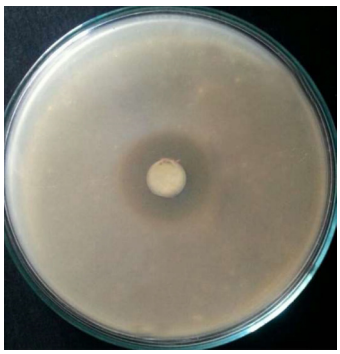

Fig. 7. Zone of inhibition of silver nanoparticles synthesized by *Pseudomonas* sp. H64 against *A. hydrophila*

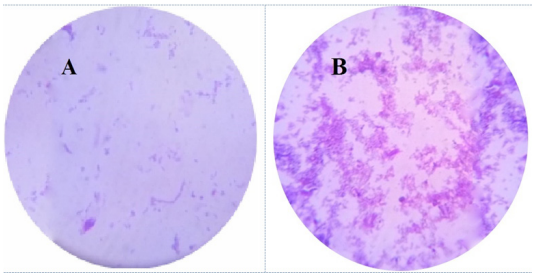

Fig. 8. Photographs showing the antifouling effect of AgNPs synthesized by *Pseudomonas* sp. H64 on biofilm formation (A), Control (without AgNPs) (B)

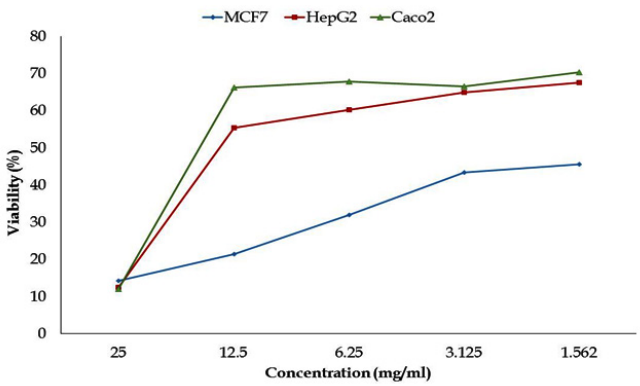

Fig. 9. Anticancer activity for different concentrations of silver nanoparticles produced by *Pseudomonas* sp. H64 against different cancer cell lines (measured by MTT assays after 24 h exposure)

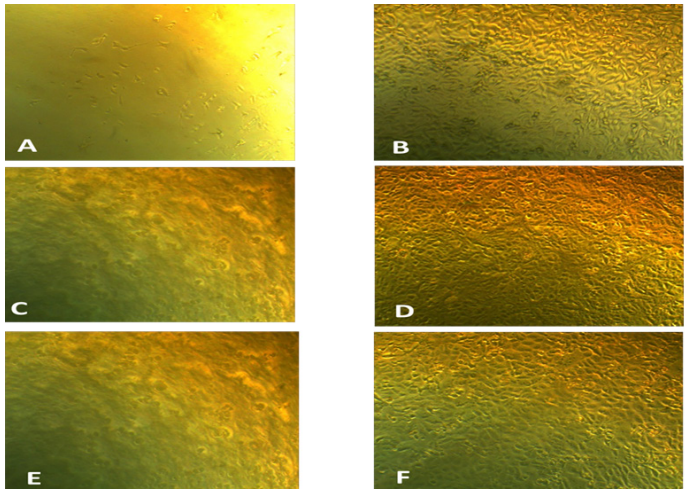

Fig. 10. Photographs illustrating the difference between the tested silver nanoparticles on growth of CaCo-2 tumor cell line (A) compared to control (B); on growth of HepG2 (C) compared to control (D) and on growth of MCV tumor cell line (E) compared to control (F)

lowest activity was against CACO. Parallel study reported 80% inhibition of MCF-7 cells with IC_{50} value less than 10 µg/ml. It was reported that the cytotoxic effect of silver nanoparticles is cell-type dependent [1] and change in morphology of the cell lines might be due to interaction between cell surface and AgNPs causing disturbance in cell composition[7].

Bioremediation study

Biosorption of synthetic Azo dyes

The biosynthesized nanoparticles by microorganisms showed excellent ecofriendly agent for catalytic activity and hence in dye effluent treatment [14]. In the present experiment the potential of silver nanoparticles in decolorization of some Azo dyes including Acid orange, Direct green, Acid black and Acid fast brown was studied. decolorization of dyes was measured using UV-Vis spectrophotometer after 2h. Results (Fig. 11) revealed that the decolorization of Acid orange, Direct green, Acid black and Acid fast brown was 90%, 64%, 59% and 52.3%, respectively. The highest decolorization was for acid orange and was confirmed by UV spectrum analysis (Fig. 12 A, B). Results (Fig. 12 A) detected the presence of

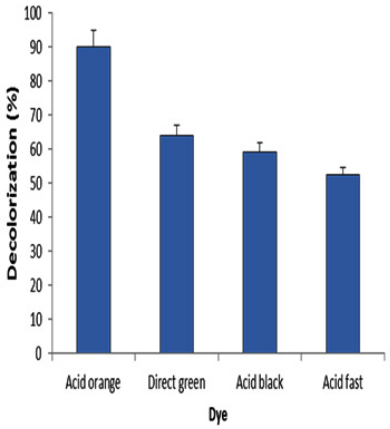

Fig. 11. Decolorization efficiency of different Azo dyes by silver nanoparticles

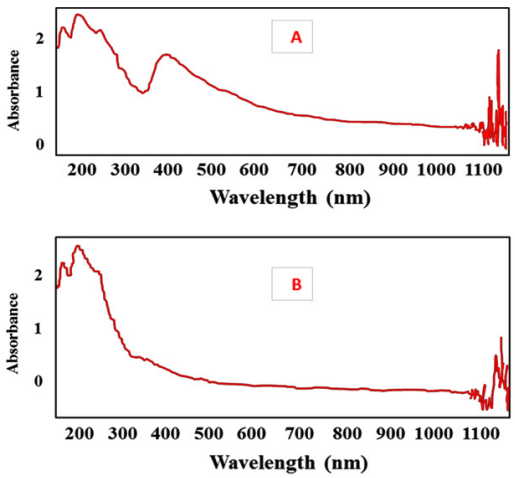

Fig. 12. UV spectra of Acid orange dye before treatment with silver nanoparticles (A); after treatment with silver nanoparticles (B)

a strong sharp peak at 420 nm in visible region of the spectrum which diminished and disappeared after 2h of treatment with AgNPs synthesized by *Pseudomonas*. sp. H64 (Fig. 12 B).The present results are in harmony with previous study[15] who showed 85% decolorization efficiency of Azo dye by Ag NPs synthesized by bacterial strain AN-1, after 32h. The highest decolorization efficiency of Phenol Red–PR, Methyl Orange–MO, Saffrain Stain Powder–SSP, Bromo Cresol Green–BCG by Ag nanoparticles synthesized using *Pterocarpus Santilanus* was 53%, 48%, 44%, 49% at the time of 30 min, 25 min, 40 min, 50 min, respectively[41]. Efficiency of Ag NPs synthesized by bacteria was also confirmed in other studies [42,43]. pH is an important factor affecting the efficiency of dye adsorption and removal by AgNPs , where at lower pH, positively charged nano particles are formed, which are favorable for removal of the negative charged dye, providing more surface area and adsorbent sites[41].

Bioremediation of heavy metals

Results (Fig. 13) indicated that the highest removal efficiency (63.5 %) by AgNPs was for Cd^{2+}, followed by 28%, 20% and 17% for Ni^{2+}, Cu^{2+} and Pb^{2+}, respectively. Al-Qahtani [44] confirmed the capability of the silver nanoparticles for removal of Pb^{2+} from aqueous solution. Also, removal efficiency for other metals including Pb^{2+}, Zn^{2+}, Cu^{2+} and Ni^{2+} was reported [45,46] showing that the removal efficiency depending on agitation speed, pH, contact time, initial metal ion concentration, and adsorption dosage. Difference in the removal efficiency for each tested metal may be also attributed to availability of binding sites on the surface of AgNPs[45].

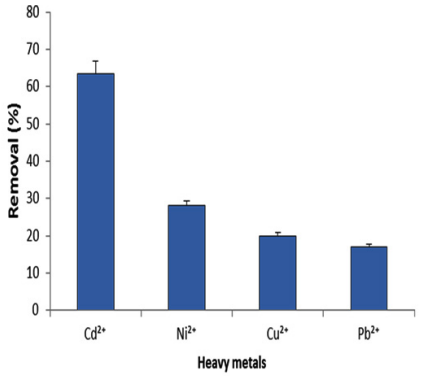

Fig. 13. Removal efficiency of different heavy metals by silver nanoparticles synthesized by marine *Pseudomonas* sp. H64

Statistical analysis

Triplicates experiments were done. Results were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The results were shown as mean ± standard deviation[47].

CONCLUSIONS

The evolution of efficient, easy and eco-friendly methods in the synthesis and application of nanoparticles are very promising and is beneficial for mankind. The present study encouraged the use of marine *Pseudomonas*. sp. H64 as natural and renewable source for biosynthesis of silver nanoparticles with different valuable applications. The biosynthesized AgNPs by *Pseudomonas*. sp. H64 could be considered as broad-spectrum antimicrobial agents. Moreover antifouling, antioxidant and antitumor activities were proven. The bioremediation potential of heavy metals and Azo dyes also was confirmed. More studies concerning the optimization of Ag NPs will be conducted in the future.

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