EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Special Issue-3 (August-2016) pp. 81-95

Journal home page: http://www.ijcrar.com

Biosynthesis of Silver Nanoparticles Using Extracellular Filtrate of Marine Bacteria and its Antimicrobial Activity

Selvakumar Dharmaraj*, Ravanappan Srinivasan Ramya, Manickam Akila, Jeevitha Ramanan and Elango Jothi Mani

PG Department of Biotechnology, Kumararani Meena Muthiah College of Arts and Science, Adyar, Chennai - 600020, Tamil Nadu, India

Corresponding author

KEYWORDS

Marine Bacteria, Extracellular synthesis, Bionanoparticles.

A B S T R A C T

The present study was focussed on the synthesis of silver nanoparticles from the extracellular components of the marine fish gut associated bacteria and its antagonistic activity. Marine fish *Cephalopholis formosa* were collected from Neelagankarai Beach, Chennai, Tamil Nadu. Two bacterial strains were isolated from gut of Marine fish and its extracellular components are used for the synthesis of bionanoparticles from silver nitrate. The characterization of nanoparticles synthesized using UV-Visible spectroscopy, SEM, and FTIR. Later they were subjected to antibacterial activities by agar well-diffusion (AWD) and disc diffusion (DD) methods against bacterial pathogens (*E. coli*, *P. aeroginosa*, *Klebsiella* sp., *S. aureus*) and fungal pathogens (*Candida albicans*, *Candida tropicalis*). Two marine strains (NDBG 01 and NDBG 02) isolated was identified as *Bacillus* sp. by conventional biochemical characterisation. The culture supernatant of upon UV-Visible spectral analysis showed absorption at 393 nm (NDBG 01) and 417 nm (NDBG 02). SEM analysis revealed to have sizes of several 23.9 to 56.1 nm (NDBG 01) and 66.7 to 215.7 nm (NDBG 02). FTIR was done to identify the biomolecules responsible for the bioreduction of silver ion and capping of the bioreduced silver nanoparticles. By AWD and DD methods exhibited the maximal inhibition zone of 10 to 15 mm against pathogens. These bionanoparticles can play a vital role in pharmaceutical industry and nano-based therapy in future.

Introduction

Nanotechnology is a fast growing area in the field of science that increased the scope of investing and regulating at cell level between synthetic material and biological system (Du *et al*., 2007; Sinha *et al*., 2009).

Nanoparticle is a core particle which performs as a whole unit in terms of transport and property. As the name indicates nano means a billionth or 10^{-9} unit.

Its size range usually from 1-100nm, due to small size it occupies a position in various fields of nano science and nanotechnology (Nour *et al*., 2010). Nano size particles are quite unique in nature because nano size increase surface to volume ratio and also its physical, chemical and biological properties are different from bulk material. So the main aim to study its minute size is to trigger chemical activity with distinct crystallography that increases the surface area (Sinha *et al*., 2009). Thus in recent years much research focussed on metals derived nanoparticles and its properties like catalyst, sensing to optics, antibacterial activity, data storage capacity (Sharma *et al*., 2009).

The biological synthesis of nanoparticle is a challenging concept which is very well known as green synthesis. The biological synthesis of nano material can solve the environmental challenges like solar energy conservation, agricultural production, catalysis, electronic, optics, and in biotechnological areas (Kumar *et al*., 2011; Evanoff and Chumanov, 2005; Soloviev, 2007). Green synthesis of nanoparticle are cost effective, easily available, eco friendly, nontoxic, large scale production and act as reducing and capping agent, when compared to the chemical method which is a very costly as well as it emits hazardous by-product which can have some deleterious effect on the environment. Biological synthesis utilizes naturally occupying reducing agent such as plant extracts, microorganisms, enzymes, polysaccharides which are simple and viable, which is the alternative to the complex and toxic chemical processes (Du *et al*., 2007).

Many microorganisms can synthesise inorganic nanoparticles like silver, gold, magnesium, cadmium sulphide and silicon oxide nanoparticles. Colloid silver nanoparticle had exhibited distinct properties such as catalytic, antibacterial (Sharma *et al*., 2009), good conductivity, and chemical stability. Silver nanoparticles have its application in the field of bio labelling, sensor, antimicrobial, catalysis, electronic and other medical application such as drug delivery (Jong and Borm, 2008) and disease diagnosis. The resistance caused by the bacterial cell for silver ions in the environment is responsible for its nanoparticles synthesis. It has been reported earlier that *Bacillus subtilis* 168 has the ability to reduce Au^{3+}ions to produce octahedral gold particles of 5–25 nm within bacterial cells by incubating the cells with gold chloride, under ambient temperature and pressure conditions (Beveridge and Murray, 1980). *Pseudomonas stutzeri* AG259 the silver resistance bacterial strain can accumulate silver nanoparticles, along with some silver sulfide, in the cell where particle size ranges from 35 to 46 nm. *Lactobacillus*, a common bacterial strain present in the buttermilk, synthesizes both Au and Ag NPs of well-defined morphology under standard conditions. Report on the synthesis of metallic nanoparticles of Ag using the cultural supernatants of *Klebsiella pneumonia*, *Escherichia coli* and *Enterobacter cloacae* (Nair and Pradeep, 2002; Shahverdi *et al*., 2007; Nair *et al*., 2010). Most of the metal ions have toxic effect on bacteria so the reduction of ions or the formation of water insoluble complexes is a defence mechanism developed by the bacteria to overcome such toxicity (Sastry *et al*., 2003). It is generally believed that the enzymes of the organisms play a key role in the bioreduction process but some studies have shown contradictory results. Previously reported that dried cells of *Bacillus megaterium* D01, *Lactobacillus sp*. A09 could reduce silver ions where the processes of bioreduction were probably non enzymatic (Fu *et al*., 2000) There silver ions

were reduce by the interaction of the silver ions with the groups on the cell wall of the microorganisms The most widely acknowledged mechanism for the biosynthesis of silver nanoparticles is the presence of the enzyme nitrate reductase which converts nitrate into nitrite. The green synthesis of silver nanoparticles from bacterial extracelluar compounds using $AgNO_3$ involves the reduction of nitrate to nitrite in the presence of Nicotinamide adenine dinucleotide (NADH) - dependent nitrate reductase. During the reduction, an electron is transferred to the silver ion; hence, the silver ion is reduced to silver (Ag^+ to Ag^0) (Prabhu and Poulose, 2012). Hence, the present study was aimed to synthesize and characterize silver nanoparticles from the extracellular components of the marine bacteria and their antagonisms against various pathogens were assessed.

Materials and Methods

Collection of Samples

The live fish of *Cephalopholis formosa* (Shaw, 1812) [Figure 1] was collected from Neelagankarai Beach, Chennai, Tamil Nadu and brought alive to the laboratory for further analysis. The fish weighs around 125 g and length of 19 cm.

Isolation of Marine fish gut associated Bacteria - Under sterile condition the fish gut was dissected which weighs around 6.8 gm and washed with normal saline (0.9% NaCl) followed by homogenization with 1 ml of saline. The homogenate was serial diluted at the rate up to 10^{-1} to 10^{-6}.

Media preparation and plating - Nutrient agar was prepared and plated with the serial dilutions (10^{-5} & 10-^{6}) in pour plate method. The plates were incubated at 37° C for overnight. Nearly 150 colonies were counted and one different colony was streaked in a separate plate and sub-cultured for further analysis.

Identification of the Strains

Morphological characterization: Two isolates (NDBG 01 and NDBG 02) were morphologically characterized by Gram's staining and motility test.

Biochemical characterization

Indole test: A loop ful of culture was inoculated into the Tryptone broth (Tryptone 10 g, NaCl 0.5 g, distilled water 100 ml, pH 7.5) and incubated at 37°C for 12 hrs. After incubation 5 drops of Kovac's reagent was added to the surface of the each culture and observed for ring formation. Appearance of Cherry red color layer ring at the top of the broth in a test tube indicates positive result. No color change of the media indicates negative result.

Methyl red - Voges Proskauer test: A loop full of culture was inoculated into the MR-VP broth (7g peptone, 5g dextrose, 5g Di-potassium phosphate, 5g NaCl, 1000 ml distilled water) and incubated at 37°C for 12 hrs. After incubation 5 drops of methyl red indicator for methyl red and 5 drops of Barritt's reagent A and B for Voges Proskauer were added. Appearance of red color indicates positive results and no color change indicates negative result.

Citrate utilization test: It was done by inoculating overnight culture into the Simmon's citrate agar slants [0.2g $(NH_4)_2SO_4$, 1.0 g $(NH_4)_2HPO_4$, 1.0 g K_2HPO_4, 0.1% $NH_4H_2PO_4$, 2.0 g sodium citrate, 5.0 g Nacl, 15g Agar, 1000ml distilled water] and incubated at 37°C for 12 hrs. If the reaction was positive, it would

give a blue color otherwise it would give the yellowish green color and also there would be no growth in the slant if it is a negative result.

Catalase test: A loop full of culture was inoculated into the slide containing a drop of hydrogen peroxide solution. Production of gas bubbles indicates positives result and their absence indicates negative.

Carbohydrate fermentation test: It was performed by inoculating the culture into the tubes containing respective sugar containing the medium (Casein enzyme digest 1g, NaCl 0.5 g, Phenol red 2 ml, Sugars 1g, distilled water100 ml and Durham's tubes were filled with broth and inverted into the culture medium incubated at 37°C for 12 hrs. The presence of gas in the Durham's tubes indicates positive result, no gas formation indicates negative.

Starch hydrolysis test: It was done by spot inoculating the culture on the starch agar plates (Nutrient agar + 1% starch) and incubated at 37°C for 12 hrs. After incubation the plates were flooded with iodine solution and allowed to stand for 5 minutes.

Nitrate reduction test: Inoculate the Nitrate broth with culture and Incubate at 370C, for 12 hrs. Add 6-8 drops of Nitrite reagent A. Add the same number of drops of Nitrite reagent B. The broth will turn a deep red within 5 minutes. If there is no color change, which indicate a negative result.

Culture and culture maintenance - Two bacterial strains isolated from gut of Marine fish were used for the study. The culture slants were maintained at 4°C in growth medium (peptone 6 g, tryptone 3g, yeast extract 3 g, beef extract 1.5g, Mn $SO_4.4H_2O$ 1mg, agar 19g in 1 l distilled H_2O, pH 7.2).

Biosynthesis of silver nanoparticles - The bacterial cultures were inoculated in growth media and incubated at 37° C for overnight. The following day the culture was centrifuged at 5000 rpm for 10mts and supernatant was collected. The collected culture supernatant of bacteria were brought in contact with 10^{-3} M $AgNO_3$ and agitated at 150 rpm for overnight in dark conditions. The following day, $AgNO_3$ treated supernatant exhibit colour change which indicates the synthesis of silver nanoparticle. Silver nitrate and other chemicals used in this study were also obtained from Hi-Media Laboratories, India.

Effect of pH on silver nanoparticles - The culture supernatant of 18 ml was mixed with 2 ml of 100 mM $AgNO_3$ and agitated at 150 rpm for overnight in dark conditions at different pH (6, 7, 8, 9 and 10). The following day colour change was noted.

Characterization of Silver Nanoparticles - The characterization study of silver nanoparticle was done by the examining size, shape and quantity of particles. Number of technique is used for this purpose, including UV-visible spectroscopy, Scanning Electron Microscopy (SEM) and Fourior Transmission Infrared Spectroscopy (FTIR).

UV-vis Spectroscopy - The synthesis of silver nanoparticles was monitored using UV-Vis spectroscopy was scanned at 300-800 nm. When the wavelength is varied and the absorbance is measured at each wavelength.

Scanning Electron Microscope - The synthesized silver nanoparticles were concentrated and powered. Scanning electron microscope (SEM) analysis was employed to characterize size, shape and morphologies of formed nanoparticle.

Fourier Transmission infrared spectroscopy - The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer (perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 75^{o} C and the dried powders were characterized in the range 4000–400 cm^{-1} using KBr pellet method. The silver nanoparticle synthesis, FTIR data measures interaction between Ag salts and proteins molecules, which accurate for the reduction of silver ions and stabilization of Ag NPS formed.

Antimicrobial assay

Agar well diffusion method - The synthesized AgNPs produced by extracellular compounds of marine bacteria were tested for their antibacterial activity against pathogens *(E.coli, P.aeroginosa, Klebsiella sp., S.aureus)* and fungal pathogens (*Candida albicans, Candida tropicalis*) by agar well diffusion method. Overnight culture of pathogenic bacteria was swabbed in Mueller Hinton agar plates. By using a sterile cork borer, wells were punctured in agar medium and one hundred microlitre of the silver nanoparticles were added to each well. The plates were then incubated at 4° C for at least 2 hours to allow the diffusion of nanoparticles followed by incubation for 24 hours at 37° C. The diameters of inhibition zones were measured. Nutrient agar and Mueller Hinton Agar used were obtained from Hi-media Laboratories and it was ready to use.

***In vitro* screening of AgNPs for antimicrobial activity (Disc method) -** The silver nanoparticle produced by extracellular compounds of probiotic bacteria of 20 µl suspensions were impregnated in sterile filter paper discs of 6 mm diameter, dried placed onto the plates previously seeded with test microorganisms. Then the plates were kept at 4° C for at least 2 h to allow the diffusion of crude extracts and incubated for 24 h at 37° C. The diameters of inhibition zones were then measured.

Results and Discussion

Two isolates (NDBG 01 and NDBG 02) from marine fish gut were obtained on nutrient agar plates. The two isolates were studied for their morphological and biochemical characterization. Morphological identification indicates that the strains were motile and Gram's positive. Biochemical characterizations were done for the two strains which are shown in Table 1. The two isolates exhibited Indole test, Catalase test, Voges proskauer test as negative, whereas Methyl red, Nitrate reduction, Starch hydrolysis test as positive. For citrate utilization test the strains NDBG 01 and NDBG 02 resulted in negative. The two strains exhibited acid positive, gas negative for all sugar test (Sucrose, Glucose, Maltose, Fructose). The cultures were tentatively identified as *Bacillus* sp. (NDBG 01 and NDBG 02) by using Bergey's Manual of Determinative Bacteriology. The fish intestine is a favorable ecological niche for microorganisms, which reach much higher numbers than in the surrounding water (Austin and Austin, 1987).

Earlier in a study nearly 50 strains were isolated from the fish intestine of black porgy fish samples, of which one isolate was considered to be probiotic bacteria according to the morphological, biochemical characteristics and metabolic products. The strain could produce acid by pH determination after fermentation, gas from glucose and dextran from sucrose, and hydrolyze arginine. The characteristics of the strain were the same as those of probiotic bacteria (Wei Zhang *et al*., 2012).

Biosynthesis of silver nanoparticles

The culture supernatant of marine gut associated bacteria on treatment with $AgNO_3$ exhibited colour change in alkaline pH (10) which was shown in Figure 2. According to previous literature, silver nanoparticle solution has dark brown or dark reddish in colour which is produced on reduction of silver ions into silver nanoparticles. This colour change is due to the property of quantum confinement which is a size dependent property of nanoparticles which affects the optical property of the nanoparticles.

UV-VIS spectrophotometer analysis - UV-vis spectroscopy is a valuable tool for structural characterization of Ag NPs. The synthesis of silver nanoparticles was monitored using UV-Vis spectroscopy which was scanned at 300-800 nm. The particles, thus produced at pH 10, showed maximal absorbance between 393 (NDBG 01) and 417 nm (NDBG 02) shown in Figure 3. It has been calibrated earlier that silver nanoparticles of size 100 nm showed maximal absorbance in the range 390-420 nm (Prabhu and Poulose, 2012). Also, it is well recognized that the absorbance of Ag NPs depends mainly upon size and shape (Kerker, 1985).

Scanning Electron Microscopy - SEM provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameters of prepared nanoparticles of strain NDBG 01 have sizes of several 23.9 to 56.1 nm and NDBG 02 have 66.7 to 215.7 in shown in Figure 4. The size of the prepared nanoparticles were less than the size of 100 nm but some nanoparticles sizes was more than the desired size as a result of the extracellular compounds which were bound to the surface of the nanoparticles (Pillai and Kamat, 2004; Chaudhari *et al*., 2007).

FTIR Analysis - FTIR gives the information about functional groups present in the synthesised silver nanoparticles for understanding their transformation from simple inorganic $AgNO_3$ to elemental silver by the action of the different phytochemicals which would act simultaneously as reducing, stabilizing and capping agent. FTIR spectrum clearly illustrates the biofabrication of silver nanoparticles mediated by the extracellular supernatant. The spectra were obtained in the wavelength range between 400 and 4000cm^{-1}. Figure 5 (A) shows the FTIR spectrum of extracellular supernatant of probiotic bacteria mediaited synthesised AgNPs peaks of NDBG 01 was observed at 3338.29 cm^{-1}, 1638.36 cm^{-1}, 1387.87 cm^{-1}, 1045.30 cm^{-1} and 627.13 cm^{-1} Figure 5 (B) shows the FTIR spectrum of extracellular supernatant of probiotic bacteria mediaited synthesised AgNPs peaks of NDBG 02 was observed at 3339.03 cm^{-1}, 1638.22 cm^{-1}, 1386.96 cm^{-1}, 1044.99 cm^{-1} and 622.91 cm^{-1}. These peak values are associated with NH stretching, C=O stretching, N-O stretching, CH_2 & CH_3 deformation, C-O stretching and halogen group presence.

These carboxyl and amide group indicate the presence of secondary amines which is a signature marker of proteins confirming the bio-fabrication of the nanoparticles by the action of the protein. The band observed at 1386.96 cm^{-1} and1387.87 cm^{-1} can be assigned to the C–N stretching vibrations of the aromatic and aliphatic amines, respectively (Swarup *et al*., 2013). These observations indicate the presence and binding of proteins with silver nanoparticles which may be the possible reason of their stabilization.

Table.1 Biochemical characterization of isolated strains (NDBG 01, NDBG 02)

Biochemical Test	Isolated Bacterial Strains	
	NDBG 01	**NDBG 02**
Indole	**-ve**	**-ve**
Methyl red	**+ve**	**+ve**
Voges proskauer	**-ve**	**-ve**
Citrate utilization	**-ve**	**-ve**
Catalase	**-ve**	**-ve**
Glucose	**-ve**	**-ve**
Sucrose	**-ve**	**-ve**
Fructose	**-ve**	**-ve**
Maltose	**-ve**	**-ve**
Starch hydrolysis	**+ve**	**+ve**
Nirate reduction	**+ve**	**+ve**
Gram's staining	**+ve**	**+ve**
Motility test	**+ve**	**+ve**

Table.2 Zone of Inhibition of the strains (NDBG 01, NDBG 02) against the selected pathogens by Agar well diffusion method

PATHOGENS	Zone of Inhibition (mm)			CONTROL
	Concentration of $AgNO_3$(µl)			
	50	75	100	
E. coli	10	13	14	-
P. aeroginosa	6	8	9	-
Klebsiella sp	10	11	13	-
S. aurues	-	-	-	-
C. albicans	-	-	-	
C. tropicalis	13	14	15	

Table.3 Zone of Inhibition of the strains (NDBG 01, NDBG 02) against the selected pathogens by Disc diffusion method

PATHOGENS	Zone of Inhibition (mm)			CONTROL (Streptomycin antibiotic disc)
	Concentration of $AgNO_3$(µl)			
	15	25	75	
E. coli	11	10	11	9
P. aeroginosa	7	8	10	7
Klebsiella sp	5	6	7	10
S. aurues	-	-	-	-
C. albicans	-	-	-	-
C. tropicalis	9	11	13	8

Fig.1 Marine fish Cephalopholis Formosa

Fig.2 Colour changes of culture supernatant of marine gut associated bacteria on treatment with AgNO3 (A – NDBG 01 and B - NDBG 02) at various pH (6, 7, 8, 9 & 10)

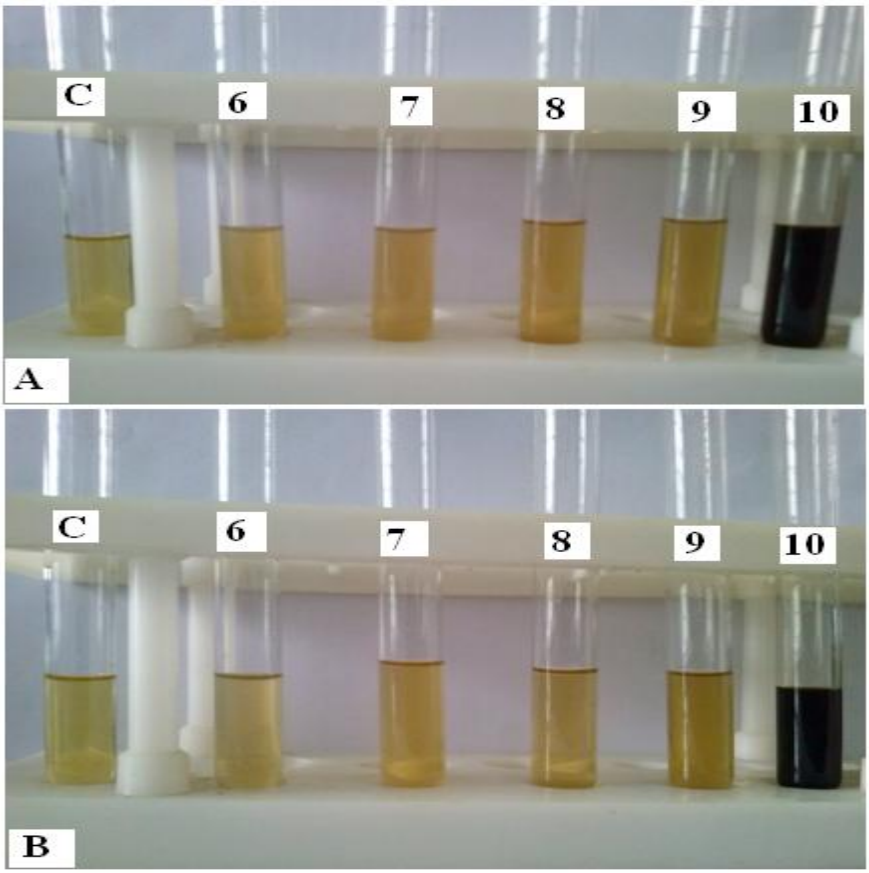


Fig.3 UV-Visible spectrum of the silver nanoparticle at pH 10 (A – NDBG 01 and B- NDBG 02) represents the samples of silver nanoparticles synthesized from extracellular components of marine gut associated bacteria

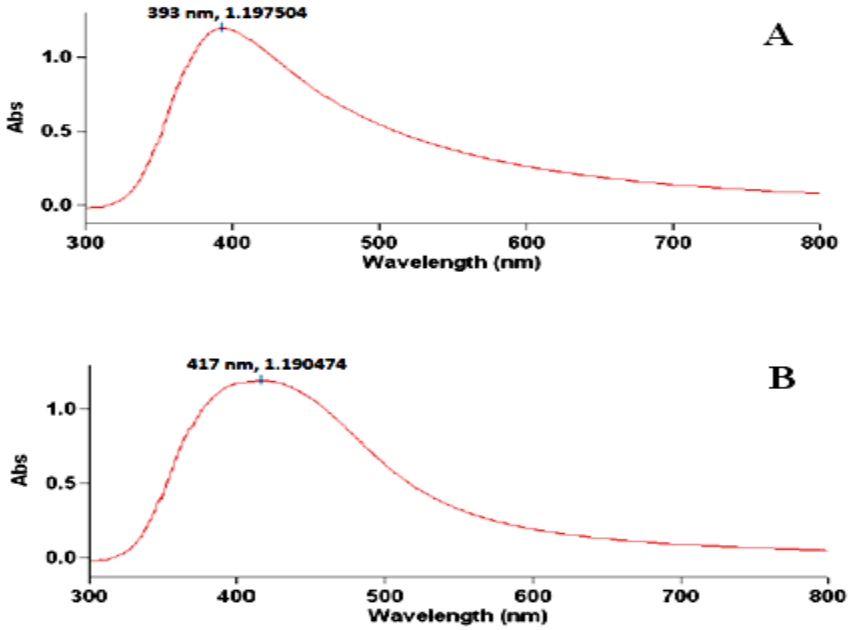


Fig.4 SEM images of Silver nanoparticles produced by extracellular compounds of A – NDBG 01 and B- NDBG 02

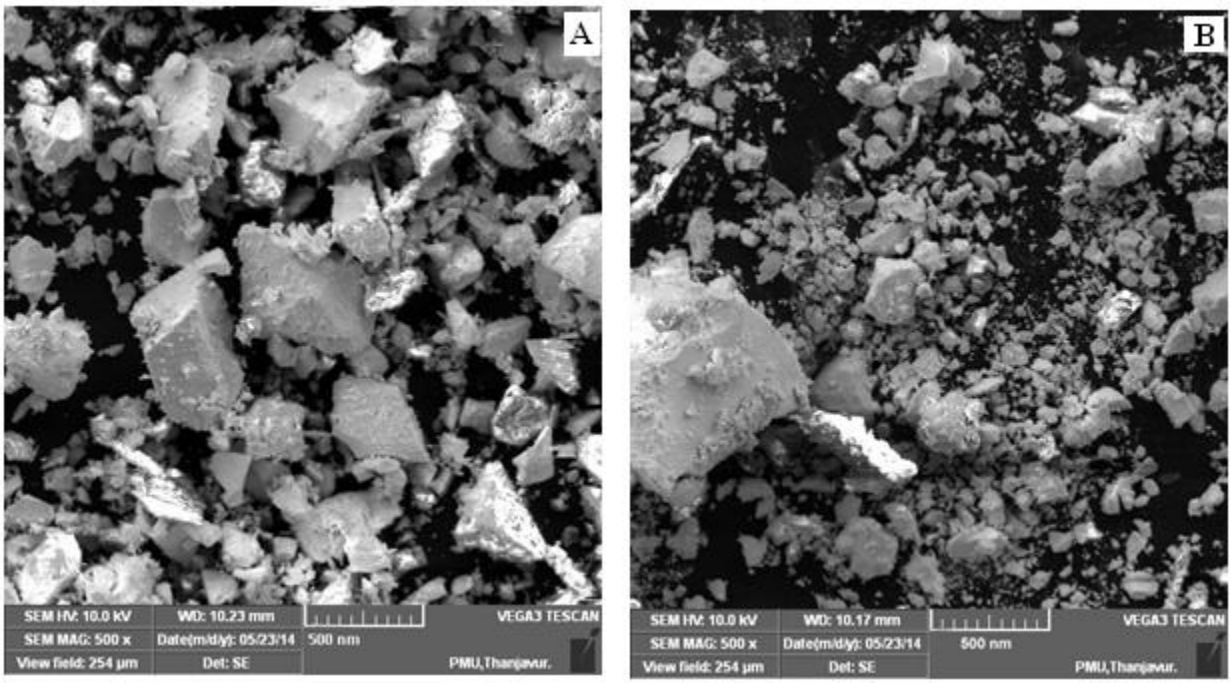


Fig.5 FTIR graph of synthesised AgNPs of A – NDBG 01 and B- NDBG 02

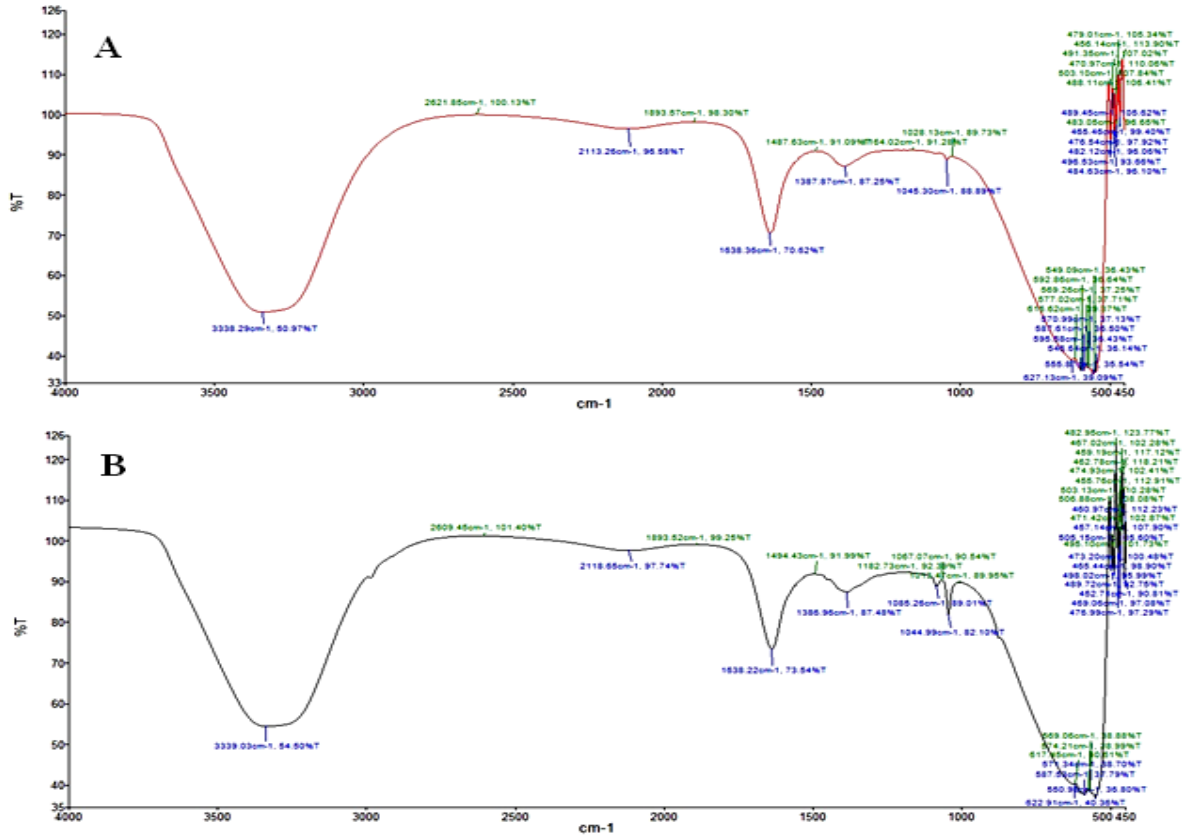
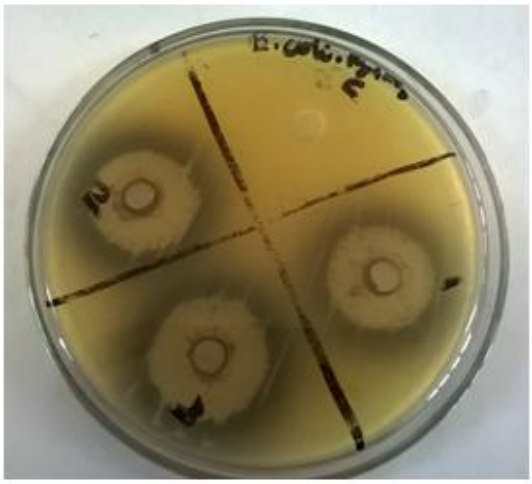
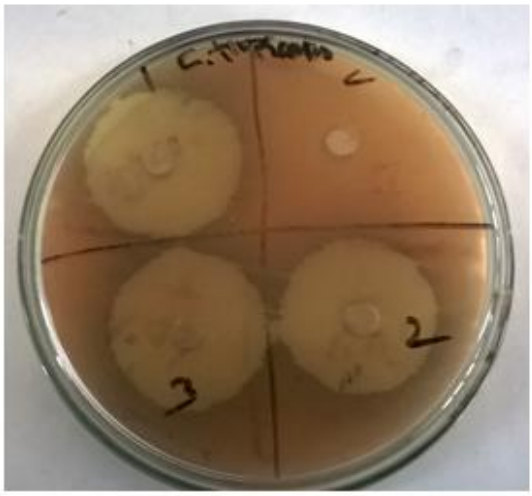


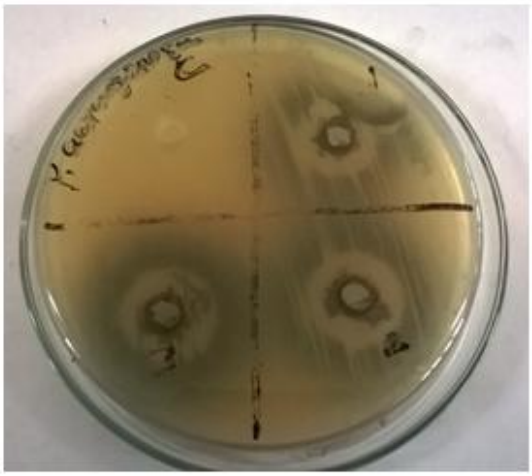
Fig.6 Zone of inhibition of nanoparticles against pathogens by Agar well diffusion method



E. coli



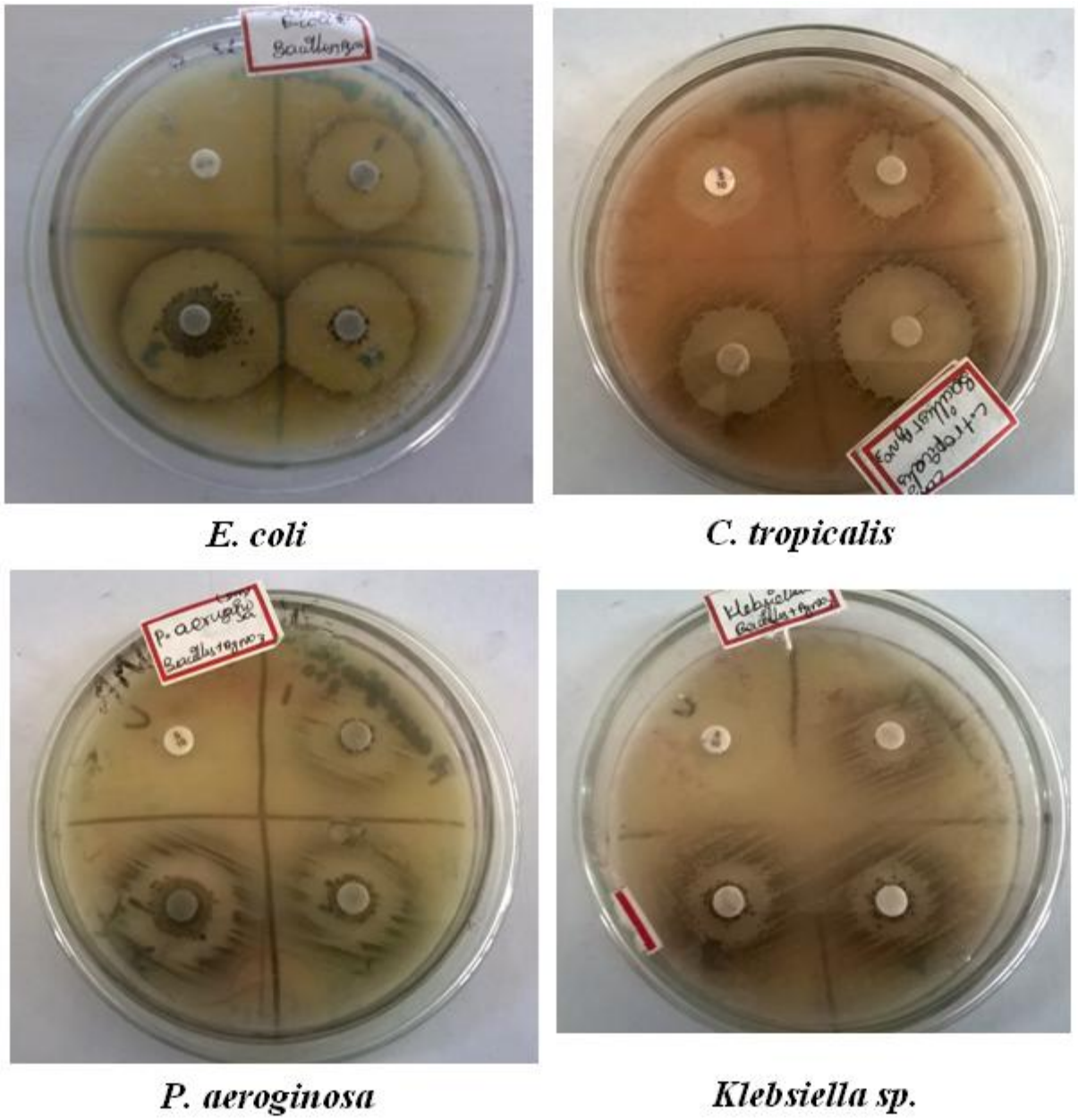
C. tropicalis



P. aeroginosa

Klebsiella sp.

Fig.7 Zone of inhibition of nanoparticles against pathogens by Disc method



Antimicrobial Study (Agar Well Diffusion Method) - The antimicrobial studies were assessed for the synthesized silver nanoparticle using agar well diffusion method against pathogens *(E.coli, P.aeroginosa, Klebsiella sp., S.aureus)* and fungal pathogens (*Candida albicans, Candida tropicalis*). Silver nanoparticles exhibited the maximal zone of inhibition of 10 to 15 mm against the pathogens which is shown in Figure 6 and Table 2. The silver antimicrobial effectiveness has been acknowledged for ages. Over the last few years, the use of silver or silver salts as key components to control the microbial proliferation has become increasingly popular. They are being currently incorporated in a wide variety of materials

used in our daily lives, which range from the textile and hospital areas to materials used in personal hygiene, such as deodorants and toothbrushes (Bouwmeester *et al*., 2009; Martinez-Abad *et al*., 2012). A recent application is based on matrixes formed by collagen and bayberry tannin for the immobilization of silver nanoparticles (AgNps) (He *et al*., 2012).

***In Vitro* Screening of Agnps For Antimicrobial Activity (Disc Method) -** The antimicrobial studies were assessed for the synthesized silver nanoparticle using disc method against pathogens. Silver nanoparticles exhibited the maximal zone of inhibition of 13 mm against the pathogens *C. tropicalis* shown in Figure 7 and Table 3. The bacterial growth was inhibited by silver ions, which accumulated into the vacuole and cell walls as granules (Brown and Smith, 1976). The silver nanoparticles attached the surface of the cell membrane disturbing the permeability and respiration functions followed by dysfunction of metabolic pathways including, silver ions can interact with nucleic acids they preferentially interact with the bases in the DNA rather than with the phosphate groups. Thereby, inhibiting the cell division and also damaged the cell envelope and cellular contents of the bacteria (Richards *et al*., 1984). In another study, the mechanism of silver nanoparticles against bacterial cells due to the sizes of the bacterial cells increased, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers exhibited structural abnormalities (Husseiny *et al*., 2015). It is also possible that silver nanoparticles not only interact with the surface of membrane, but can also penetrate inside the bacteria. When the silver nanoparticles enters into the bacteria that, generating hydrogen peroxide radicals followed by inactivated metabolic enzymes, which leads bacterial cell death.

Conclusion

Microbial synthesis of nanoparticles has been emerged as an important branch of nano biotechnology. Due to their rich diversity, microbes have the innate potential for the synthesis of nanoparticles and they could be regarded as potential biofactories for nanoparticles synthesis. Addition to microorganisms, their component can use for nanoparticle synthesis. Some biological molecules like fatty acids, amino acids, are used as template in the growth of semiconductor nano-crystals. Biological materials like DNA, protein, biolipid cylinders, viroid capsules, S-layers and multicellular superstructures have been used in template-mediated synthesis of inorganic nanoparticles. Although to improve synthesis rate and monodispersity of nanoparticles, factors such as microbial cultivation methods and downstream processes techniques should be heal and combination of methods may be used such as photobiological methods, describe specific genes and characteristization of enzymes involved in the biosynthesis of nanoparticles is also required. Therefore, a complete knowledge of the molecular mechanisms involved in the microbial mediated synthesis of nanoparticles is neseccery to control the size, shape and crystallinity of nanoparticles. A green chemistry synthetic route has been used for silver nanoparticles synthesis. Analytical techniques, such as Ultraviolet-Visible spectroscopy (UV-vis), X-ray powder diffraction (XPD), Transmission electron microscopy (TEM) and Zeta potential measurements were applied to characterize the nanoparticles morphology.

Silver nanoparticles have a number of applications from electronics and catalysis to biology, pharmaceutical and medical diagnosis and therapy. The antibacterial

activity of silver ions is well known, however, the antibacterial activity of elementary silver, in the form of nanoparticles has been developed. Nanoscale drug delivery systems have the ability to improve a distribution of medicines. The nanoparticles were utilized to facilitate the specific interactions between anticancer drugs and DNA. This may create a valuable application of metal nanoparticles in the relative biomedical area.

References

Austin, B., and Austin, D.A. 1987. Bacterial fish pathogens: Disease in farmed and wild fish. Ellis Horwood Ltd., West Sussex, England, pp. 13-350.

Beveridge, T.J. and Murray, R.G.E. 1980. Site of metal deposition in the cell wall of *Bacillus subtilis*. *J. Bacteriol*., 141:876–887.

Bouwmeester, H., Dekkers, S., Noordam, M.Y., Hagens, W.I., Bulder, A.S., De Heer, C., Ten Voorde, S.E.C.G., Wijnhoven, S.W.P., Marvin, H.P.J and Sips, A.J.A.M. 2009. Review of health safety aspects of nanotechnologies in food production. *Regul. Toxicol. Pharmacol*., 53:52–62.

Brown, T and Smith, D.1976. The effects of silver nitrate on the growth and ultrastructure of the yeast *Cryptococcus albidus. Microbios. Lett*., 3, 155-162.

Chaudhari, V.R., Haram, S.K and Kulshreshtha, S.K. 2007. Micelle assisted morphological evolution of nanoparticles. *Colloids. Surf A*., 301:475-80.

Du, L., Jiang, H., Liu, X and Wang, E. 2007. Biosynthesis of gold nanoparticles assisted by *Escherichia coli* DH5a and its application on direct electrochemistry of haemoglobin. *Electrochem. Commun*., 9:1165-1170.

Evanoff, D.D and Chumanov, G. 2005. Synthesis and Optical Properties of Silver Nanoparticles and Arrays. *Chem. Phys. Chem*., 6:1221–1231.

Fu, M., Li, Q., Sun, D., Lu, Y., He, N., Deng, X., Wang, H and Huang, J. 2006. Rapid Preparation Process of Silver Nanoparticles by bioreduction and their characterizations. *Chinese J. Chem. Eng*., 14(1):114-117.

He, L., Gao, S.Y., Wu, H., Liao, X.P., He, Q and Shi, B. 2012. Antibacterial activity of silver nanoparticles stabilized on tannin-grafted collagen fiber. *Mater. Sci. Eng.C*., 32:1050–1056.

Husseiny, S.M., Salah, T.A and Anter, H.A. 2015. Biosynthesis of size controlled silver nanoparticles by *Fusarium oxysporum*, their antibacterial and antitumor activities. *Beni-Suef University. J. Basic. Appl. Sci*., 4: 225-231.

Jong, W.H.D and Borm, P.J.A. 2008. Drug delivery and nanoparticles: Applications and hazards. *Int. J. Nanomed*., 3 (2): 133–149.

Kerker, M. 1985. The optics of colloidal silver: something old and something new. *J. Colloid. Interface. Sci*., 105:297-314.

Kumar, B., Naik, V., Halehatty, S.B., Girija, D and Kumar, B. V. 2011. ZnO nanoparticle as catalyst for efficient green one-pot synthesis of coumarins through Knoevenagel condensation. *J. Chem. Sci*., 123: 615-62.

Martinez-Abad, A., Lagaron, J.M and Ocio, M.J. 2012. Development and characterization of silver-based antimicrobial ethylene−vinyl alcohol copolymer (EVOH) films for food-packaging applications. *J. Agric. Food. Chem*., 60(21):5350–5359.

Nair, B and Pradeep, T. 2002. Coalescence of nanoclusters and formation of

submicron crystallites assisted by *Lactobacillus* strains. *Crystal Growth. Design*., 2: 293- 298.

Nair, R., Varghese, S.H., Nair, B.G., Maekawa, T., Yoshida, Y and Kumar, S.D. 2010. Nanoparticulate material delivery to plants. *Plant. Sci*., 179:154–163.

Nour, A.E., Kholoud, M.M., Eftaiha, A., Reda A.A.W and Ammar, A.A. 2010. Synthesis and applications of silver nanoparticles. *Arab. J. Chem*., 3: 135–140.

Pillai, Z. S and Kamat, P.V. 2004. What factors control the size and shape of silver nanoparticles in citrate ion reduction method? *J. Phys. Chem B*., 108:945-51.

Prabhu, S and Poulose, E.K. 2012. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *Int. Nano. Lett*., 2(32):1-10.

Richards, R., Odelola, M. E and Anderson, B. 1984. Effect of silver on whole cells and spheroplasts of a silver resistant *Pseudomonas aeruginosa*. *Microbios*., 39:151-157.

Sastry, M., Ahmad, A., Khan, M.I and Kumar, R. 2003. Biosynthesis of metal nanoparticles using fungi and actinomycete. *Curr. Sci*., 85:162–170.

Shahverdi, A.R., Minaeian, S., Shahverdi, H.R., Jamalifar, H and Nohi, A. A. 2007. Rapid synthesis of silver nanoparticles using culture supernatants of *Enterobacteriaceae*: A novel biological approach. *Process. Biochem*. 42: 919- 923.

Sharma, K.V., Yngard, A.R and Lin, Y. 2009. Silver nanoparticle: Green synthesis and their antimicrobial activities. *Adv. Colloid and Interface Sci*., 145: 83–96.

Sinha, S., Pan, I., Chanda, P and Sen, S.K. 2009. Nanoparticles fabrication using ambient biological resources. *J. Appl. Biosci*., 19: 1113-1130.

Soloviev, M. 2007. Nanobiotechnology today: focus on nanoparticles. *J. Nanobiotechnology*., 5(11): 1-3.

Swarup, R., Ttriparna, M., Shatarupa, C and Tapan, K.D. 2013. Biosynthesis, characterisation and antifungal activity of silver nanoparticles synthesized by the fungus *Aspergillus foetidus* MTCC8876. *Digest. J. Nanomat. Biostru*., 8 (1):197 – 205.

Zhang, W., Liu, M and Dai, X. 2012. Biological characteristics and probiotic effect of *Leuconostoc lactis* strain isolated from the intestine of black porgy fish. *Brazil. J. Microbiol*., 44: 685-691.

How to cite this article:

Selvakumar Dharmaraj, Ravanappan Srinivasan Ramya, Manickam Akila, Jeevitha Ramanan, Elango Jothi Mani. 2016. Biosynthesis of Silver Nanoparticles Using Extracellular Filtrate of Marine Bacteria and its Antimicrobial Activity. *Int.J.Curr.Res.Aca.Rev. Special Issue-3*: 81-95.