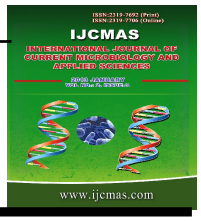
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7692 **Volume 2 Number 3 (2013) pp.57-64**
http://www.ijcmas.com



Original Research Article

Biosynthesis of Silver Nanoparticles from *Corynebacterium* sp. and its antimicrobial activity

P.Arun[1*], V.Shanmugaraju[1], J.Renga Ramanujam[2], S.Senthil Prabhu[2] and E.Kumaran[2]

[1]Department of Biotechnology, Dr.N.G.P.Arts and Science College, Coimbatore- 641 048,
[2]Department of Microbiology, Dr.N.G.P.Arts and Science College, Coimbatore- 641 048.
**Corresponding Author: arunbiotech2000@gmail.com*

K e y w o r d s

Antibacterial activity; *Corynebacterium* sp.*;* EDAX; Silver Nanoparticle; SEM.

A B S T R A C T

Nanoparticles are at the leading edge of the rapidly developing field of nanotechnology. The synthesis of nanoparticles of specific size and composition is the burgeoning area in the nanotechnology research. Advances in the field of nanotechnology largely depend on the ability to synthesize nanoparticles of various material sizes and shapes as well as to efficiently assemble them into complex architecture. In this study silver nanoparticles were synthesized using *Corynebacterium* sp. The formation of silver nanoparticles was confirmed by UV-Visible spectrophotometer and their characterization was done by SEM and EDAX analysis. The antibacterial activity of the synthesized silver nanoparticles was checked against some human pathogenic bacteria.

Introduction

Nanotechnology has been defined as a technology that mainly consists of the process of separation, consolidation and deformation of materials by one atom or molecule (Taniguchi 1974). Nanotechnology is currently fast growing niche in the field of nanoscience (Mandal *et al*., 2006). Nanotechnology is an emerging field in the area of interdisciplinary research, especially in biotechnology. One of the major challenges of current nanotechnology is to develop reliable experimental protocols for the synthesis of nanoparticles over range of chemical composition, size and synchronized monodispersity that must also be nontoxic, clean, ecofriendly by using ambient biological sources. Nanoparticles have been synthesized (Jana *et al.*, 2001) but the quest is still on for easier and commercially viable methods for the production of important nanoparticles. The importance of biological synthesis is being emphasized globally at present because chemical methods are capital intensive, toxic, non eco-friendly and have low productivity (Kowshik *et al*., 2003). The synthesis of the nanoparticle is one of the most interesting fields of research.

Silver exhibits the highest efficiency of Plasmon excitation (Kreibig *et al*., 1995). Moreover, optical excitation of plasma resonance in silver nanoparticle is the most efficient mechanism by which light interacts with matter. A single silver nanoparticle interact with light more efficiently than a particle of the same dimensions composed of any known organic or inorganic chromophore. The light interaction cross-reaction for silver can be about ten times that of the geometric cross-section, which indicates that the particle capture much more light than the physically incident on them (Evanoff *et al*., 2004; Kelly *et al*., 2003). Silver is also the only material whose plasmon resonance can be tuned to any wavelength in the visible spectrum.

The properties of these silver nanoparticles in applications as diverse as catalysis, sensors and medicine depend critically on the size and composition of the nanoparticles. Researchers in the area of nanoparticles synthesis are mainly oriented in controlling their shape, size and compositions. Each of these parameters is an important factor in determining the properties of nanoparticles that lead to various technological applications (Chandross *et al*., 1999; Djalali *et al*., 2004). Nanoparticles can be employed in a great number of applications such as fuel cells, heterogenous catalysts, electrocatalysts, and pigments. Silver nanoparticles are antimicrobial towards a broad spectrum of Gram negative and Gram positive bacteria (Kim *et al*., 2007; Panacek *et al*., 2006).

Furthermore silver nanoparticles show antifungal and antiviral activity (Elechiguerra *et al*., 2005, Lu *et al*., 2008).Besides their antimicrobial properties, silver nanoparticles can be used for their catalytic, conductive and optical features (Medina et al., 2005; Fuller *et al*., 2002).

The noble metals especially silver have attracted great attention due to their innumerable applications in various branches of science namely catalysis (Lewis *et al*., 1993), photonics (Maier *et al*., 2001;Carotenuto *et al*., 2001), photography (Lam *et al*., 1991), chemical sensing (Kim *et al*., 2001). Surface Enhanced Raman Scattering is mostly important in the medicinal fields as anti-microbial agents (Malaiye *et al*., 2005). In the current study, silver nanoparticles were synthesized from *Corynebacterium* sp. and their antibacterial activity was checked against different clinical pathogens.

Materials and Methods

Cultures

Corynebacterium sp. was obtained from Micro Lab, Coimbatore. Pure cultures of *Escherichia coli, Corynebacterium diphtheria, Klebsiella pneumoniae, Bacillus* sp. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from KMCH, Coimbatore.

Synthesis of silver nanoparticles from bacterial filtrate

The culture was centrifuged at 5000 rpm for 15 minutes and the supernatant was taken as bacterial filtrate. 10 ml of bacterial filtrate was mixed with 90 ml of silver nitrate solution (1mM) and incubated in a brown bottle for 3 days at room temperature. The metal processed bacterial filtrate was centrifuged at 8000

rpm for 20 minutes. The semi-solid pellet was dried and powdered using petroleum ether. Few drops of petroleum ether was added to the semi-solid pellet and kept for vaporization. The powdered pellet was stored at 4^{o}C.

UV-Visible spectrophotometric analysis

Qualitative testing of the supernatant was done by UV-Visible spectrophotometer to confirm the reduction of silver ions. The Silver nitrate solution was kept as control and 1ml of sample supernatant was withdrawn after 24 hr and absorbance was measured by using UV-Visible spectrophotometer between 400-600 nm.

Nitrate reductase assay

Nitrate reductase is an enzyme that converts nitrate to nitrite. The enzyme activity was measured using the method described by Harley (1993). The activity was measured by adding the substrate for the enzyme (nitrate) and then measuring the amount of nitrite after 1hr. The net increase in nitrite at 1hr is the amount of nitrate reductase activity. 0.5 ml of phosphate buffer was pipette out into the test tubes, in that 0.2 ml of standard potassium nitrate solution was added and mixed well. 0.4 ml of NADH and 0.7 ml of distilled water was added to the tubes. To this mixture 0.2 ml of bacterial filtrate - silver nitrate mixture was added and the control was maintained without the bacterial filtrate – silver nitrate mixture. The tubes were incubated in a water bath at 30^{o}C for 15 minutes. The reaction mixture was then terminated by adding 1 ml of NED reagent and then the tubes were incubated for 90 minutes. The OD values were observed at 540 nm in regular intervals of time.

Characterization of silver nanoparticles

SEM analysis:

Scanning Electron Microscopic (SEM) analysis was carried out in Karunya University, Coimbatore, using Hitachi S-4500 SEM machine. Thin films of the sample was prepared on a carbon coated grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

EDAX Analysis

In order to carry out EDAX analysis, the extracts reduced silver Nanoparticles were dried and drop coated on to carbon film and performed on Hitachi S-3400 NSEM instrument equipped with a Thermo EDAX attachments.

Antibacterial activity of silver nanoparticles

The antibacterial activity of the synthesized silver nanoparticles was checked against both Gram positive and Gram negative bacterium. Agar well diffusion method was done to determine the antibacterial activity of the synthesized silver nanoparticles against human pathogenic bacteria *viz., Escherichia coli, Corynebacterium diphtheriae, Klebsiella pneumoniae, Bacillus* sp., *Pseudomonas aeruginosa and Staphylococcus aureus.* These organisms were grown in nutrient broth for 24 hr. Approximately 20 ml of Muller Hinton agar was poured in sterilized Petri dishes and allowed to solidify.

The plates were swabbed with the organisms. Agar wells of 5 mm diameter were prepared with the help of a sterilized stainless steel cork borer. Five wells were prepared in the agar plates. The wells were labeled as E, E1, D, D1 and N1. The synthesized silver nanoparticles were dissolved in ethanol, DMSO, distilled water and added to the wells completely. Ethanol and DMSO were also maintained as control separately in the wells. The plates which were inoculated with the bacterial and silver nanoparticles were incubated at 37^{o}C for 24 hr. The plates were examined for evidence of clear zones of inhibition. The diameter of such zones of inhibition was measured using a meter ruler and the values for each organism was recorded and expressed in millimeter.

Result and Discussion

Synthesis of silver nanoparticles

Cell free filtrate of *Corynebacterium* sp. was mixed with silver nitrate solution and incubated in dark in rotary shaker for 72 hours. Samples showed change in colour from almost colourless to brown, this is a clear indication of the formation of silver nanoparticles in the reaction mixture. The intensity of the colour was increased during the period of incubation. The appearance of brown colour was due to the excitation of surface Plasmon vibrations. The control showed no change in colour of the mixture when incubated in the same conditions. (The colour change is shown in the Fig.1)

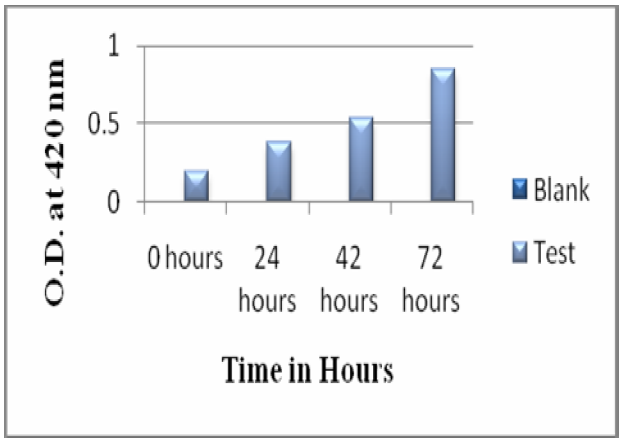
UV-VIS spectrophotometric analysis

Synthesis of colloidal silver nanoparticles was initially performed by UV-Visible spectroscopic analysis. In UV-Visible spectrum, a strong peak was observed between 420 nm, indicating the presence of silver nanoparticles. (The OD values are shown in the Table.1; Fig.2.)

Table. 1 OD values for UV-VIS Spectrophotometric analysis

	0 hours	**24 hours**	**42 hours**	**72 hours**
Blank	0.00	0.00	0.00	0.00
Test	0.19	0.38	0.53	0.85

Figure.2 OD values of UV Spectrophotometer



Nitrate reductase assay

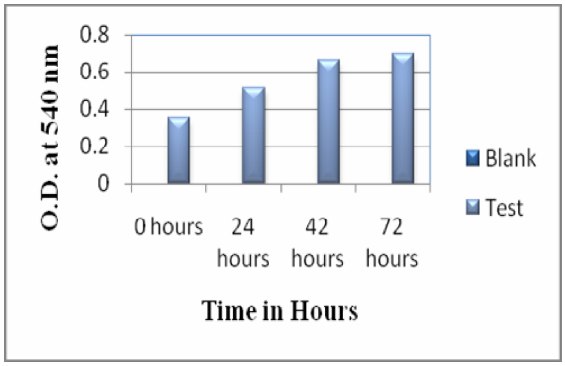
In this study, the nitrate reductase activity and the presence of nitrate reductase enzyme in the culture filtrate of *Corynebacterium* sp. was detected by nitrate reductase assay. Hence, nitrate reductase activity and the presence of nitrate reductase enzyme in the isolate indicates the reduction of silver nitrate into

silver nanoparticles. (The OD values are shown in the Table.2; Fig.3).

Table. 2 OD values for nitrate reductase Assay

	0 hours	24 hours	42 hours	72 hours
Blank	0.00	0.00	0.00	0.00
Test	0.35	0.51	0.66	0.70

Figure.3 OD values for nitrate reductase assay



Characterization of silver nanoparticles

SEM analysis

The SEM micrographs of nanoparticles obtained in the filtrate showed silver nanoparticles with an average size of about 24.55 nm, 24.46 nm, 24.85 nm, 25.34 nm, 26.76 nm, 31.58 nm, 32.05 nm (Fig. 4).

EDAX analysis

Analysis through Energy dispersive X-ray (EDX) spectrometers confirmed the presence of elemental silver signal of silver nanoparticles. The vertical axis displays the number of X-ray counts with the horizontal axis displays energy in Kev. Identification lines for the major emission energies for silver (Ag) are displayed and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified (Fig. 5)

Figure. 4 SEM analysis of Nanoparticles

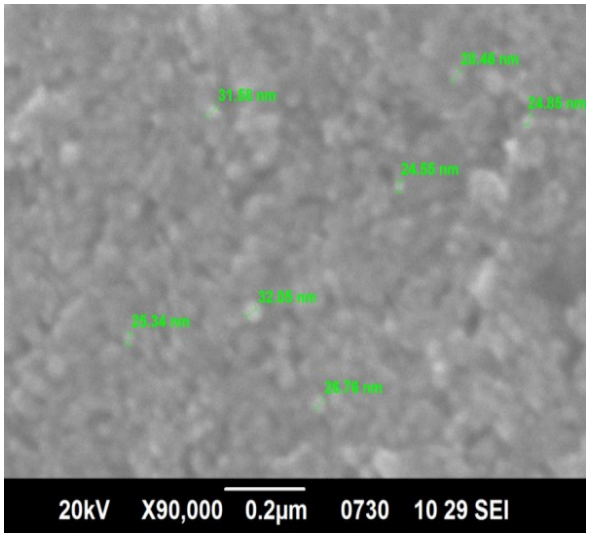
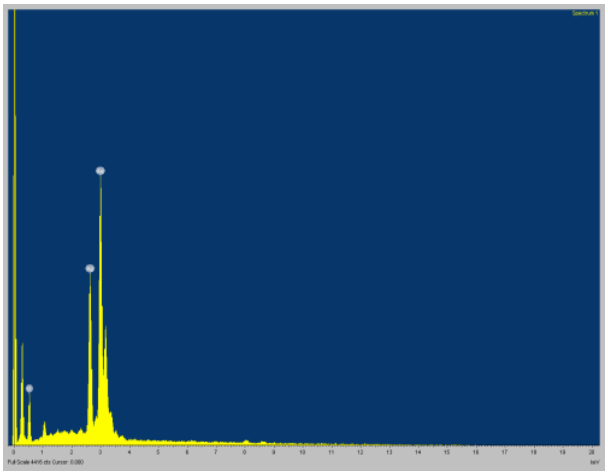


Figure. 5 EDAX analysis of nanoparticles



Antibacterial activity of silver nanoparticles

The synthesized silver nanoparticles showed activity against *Corynebacterium*

diphtheriae, Escherichia coli, Bacillus sp., *and Staphylococcus aureus* and no activity was shown against *Pseudomonas aeruginosa*. The activity was shown with the presence of zone of inhibition. The synthesized silver nanoparticles showed more activity in ethanol dilutions of *Staphylococcus aureus, Escherichia coli, Bacillus* sp. *and Corynebacterium diphtheriae* (Table. 3; Fig. 6 - 10).

Table. 2 Antibacterial activities of silver nanoparticles synthesized from *Corynebacterium* sp. against different clinical pathogens

Test organisms	Zone of Inhibition (mm)				
	Ethanol	**DMSO**	**Nanoparticles (Ethanol) 100mg**	**Nanoparticles (DMSO) 100mg**	**Nanoparticles (Water) 100mg**
Bacillus sp	5	6	9	8	10
Staphylococcus aureus	6	7	13	11	11
Pseudomonas aeruginosa	4	5	-	-	-
Escherichia coli	5	5	10	9	9
Corynebacterium diphtheriae	6	5	7	8	9

The synthesis, characterization and applications of biologically synthesized nanoparticles have become an important branch of nanotechnology. In this study silver nanoparticles were synthesized using *Corynebacterium* sp. The formation of silver nanoparticles was confirmed by UV-Visible spectrophotometer and their characterization was done by SEM and EDAX analysis.

The antibacterial activity of the synthesized silver nanoparticles was checked against different clinical pathogens like *Escherichia coli, Corynebacterium diphtheriae, Klebsiella pneumoniae, Bacillus* sp., *Pseudomonas aeruginosa and Staphylococcus aureus.* Antibacterial activity of silver nanoparticles has maximum effect on *Staphylococcus aureus* compared to other bacterial pathogens. This biological approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other nanoparticles.

References

Jun Sung Kim., Eunye kuk, Kyeong Nam Yu,Jong –Ho kim, Sung Jin Park, Hu jng Lee, So Hyun Kim and Young Kyung Park. 2007. Antimicrobial effects of Silver Nanoparticles, J. Nanomedcine. :3:95-101.

Kowshik, M., S. Ashtaputre S. Khaeeazi W. Vogel, J. Urban, S.K. Kulkarni and Paknikar, K.M. 2003. Extracellular synthesis of silver nanoparticles. J. Mat. Chem. (17): 2459-2464.

Kreibig, U., and Vollmer, M. 1995. Optical properties of metal clusters, Springer series in material science 25, New York, Springer-Verlag.pp. 32-35.

Mandal, D., M.E. Bolander, D. Mukhopadhy, G. Saikar and Mukherjee, P. 2006. The use of microorganism for the formation of metal nanoparticles and their application,.Appl. Microbial. Biotechnol.69:485-492.

Minaeian, S., A.R. Shahverdi, A.S. Nohi and Shahverdi, H. R.2008. Extracellular Biosynthesis of Silver Nanoparticles by some bacteria, JSIAU.17(66):1-4.

Mritunjai Singh., Shinjini Singh, S. Prasad and Gambir, I. S. 2008. Nanotechnology in medicine and antibacterial effect of silver nanoparticles, Digest. J. Nanomat. Biostruc. 3(3):115-122.

Nalenthran Pugazhenthiran., Sambandam Anandan, Govindarajan Kathiravan, Nyayiru Kannaian Udaya prakash, Simon Crawford, Muthupandian Ashokkumar. 2010. Microbial synthesis of Silver Nanoparticles by *Bacillus* sp. J. Nanopar. Res. 1811-1815.

Nelson Duran. , D. Priscyla, Marcatod, L. Oswaldo, P. Alves, Joao, S. Dasilva, I. Gabriel and De Souza H. 2010. Ecosystem protection by effluent bioremediation: silver nanoparticles impregnation in a textile fabrics process, J. Nanopar. Res. 285 – 292.

Nikolay Krumov., Iris Perner-nochta, Stephanie Oder, Velitchka Gotcheva, Angel Angelov and Clemens Posten, 2009. Production of inorganic Nanoparticles by Microorganisms, J. Chem. Eng. Technol. 32(7):1026-1035.

Nithiya, R,and Ragunathan, R. 2009. Synthesis of Silver nanoparticle using *Pleurotus sajor caju* and its Antibacterial activity. Digest. J. Nanomat. Biostruc.4(4):623-629.

Panacek, A. K.. L.Vitek, R. Prucek, M. Kolar, R.Vecerova, N.Pizurova, V.K.Sharma, T. Neveena and Zboril, R. 2006. Silver colloid nanoparticles synthesis, characterization and their antibacterial activity. J. Phy. Chem. 110:16248-16253.

Qilin Li, Shaily Mahendra, Delina Y Lyon Brunet, Michael V Liga, Dong Li, Pedro J J Aluarez .2008. Antimicrobial Nanomaterials for water disinfection and microbial control: Potential applications and implications. Water. Res. 4591 – 4602.

Shirley., A. Dayanand, B. Sreedhar, G. Syed and Dastager. 2010. Antimicrobial Activity of Silver Nanoparticles Synthesized from Novel *Streptomyces* species. Digest. J.Nanomat. Biostruc. 5(2): 447-451.

Siddartha Shrivastava., Tanmay Bera, Arnab Roy, Gajendra Singh, P. Ramachandrarao and Dash. 2007. Characterization of enhanced antibacterial effects of novel silver nanoparticles, Nanotechnology.

Singh, M., S. Singh, S.Prasad and Gambhir. 2008. Nanotechnology in medicine and antibacterial effect of silver nanoparticles. Digest. J. Nanomat. Biostruc. 3: 115 – 122.

Sougata Sarlcar, Atish Diparkar Jana, Samir Kumar Ssamanta, Golam Molstafa, 2007. Facile synthesis of silver nanoparticles with highly efficient anti-microbial property, Polyhedron. 26:4419-4426.

Taniguchi, N.,1974. "On the Basic Concept of 'Nano-Technology'," Proc. Intl. Conf. Prod. Eng. Tokyo, Part II, Japan Society of Precision Engineering.

Tetsuya Osaska, Tadashi Matsunanga, Takoya Nakanishi, Atsushi Arakaki, Daisuke Nicoa and Hironori Iida, 2006. Synthesis of magnetic nanoparticles and their application to bio assay vol:384, 593-600.

Thirumurugan, G., S.M. Shaheedha, Dhanaraju, M.D. 2009. *In-vitro* evaluation of antibacterial activity of silver Nanoparticles synthesized by using *Phytophthora infestans.* Int. J. Chem.Tech. Research.1(3):714-716.

Vijay ,C., Verma, N.Ravindra Kharwar and Alan C Gange. 2010. Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus*. Nanomedicine. 33- 40.