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RESEARCH ARTICLE

COMPARATIVE STUDY ON THE BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES BY E.*COLI* USING LB AND M9 MEDIA AND THEIR ANITIMICROBIAL APPLICATION

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ABSTRACT

Nanobiotechnology, a new exploration gives rise to the emerging future of science bygone decade. It covers enormous technical branches like biotechnology, chemical, medical and robotic engineering. Silver and silver-constructed product influence burly bacterial and fungal activity. In the present work, a study which compares the synthesis of silver nanoparticles (AgNPs) from *E.coli* using two different media, LB and M9 medium respectively. The influence of the parameters kind of time, substrate concentration and pH on the synthesis of nanoparticles were studied.The synthesized nanoparticles were characterized and showed antimicrobial on well diffusion assay.

Key words: Anti-microbial, *E.coli,* LB broth, M9 medium, Nanocrystalline materials, Nano sized Particles, silver nanoparticle

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INTRODUCTION

Nanotechnology is expeditious developing field which is based on the construction of functional materials, system and devices etc. These devices and system are been controlled by atoms on the nanoscale which express a novel properties in the particular size. Its influence on wealth and health of the people with the minimal equivalent to the different fields such as computer engineering, microelectronics, polymers synthesized by artificial methods and medical imaging (Anisa Mnyusiwalla *et al*., 2003). Nanoparticles are extensive materials that comprise particulate substance which are less than100 nm in size. This can influence the physio-chemical properties of a substance such as optical properties due to their size (Ibrahim Khan *et al*., 2017). The nitrate reductase is a membrane bounded respiratory enzyme. These enzyme are of three kinds in that most commonly two are known NR_A and NR_z. These enzyme is been produced by *E.coli* in the absence of free oxygen. NR_A - 98% responsible for this activity where as the NR_z part remains unknown (Bonnefoy *et al*., 1994). Sliver nitrate with β-NADH reduced into nitrite, β-NAD and water molecule by nitrate reductase. The nitrite from the above reaction reacts with sulphanilamide and NED (napthylethylenediamine) which produces nitrite a brown colour complex (Bahareh Khodashenas *et al*., 2005). The synthesis of nanoparticles are of different types - chemical, physical and biological routes. The chemical and physical routes has a negative impacts on both environment and humans therefore the ultimate way is biological route. Silver nanoparticles has an incomparable effect pathogenic microbes particularly in *E.coli* and *B.subtilis.* The synthesis of AgNPs takes place by reducing $AgNo_3$ by nitrate reductase in the presence of sun light (i,e) light reaction for a particular bout of time.

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MATERIALS AND METHODS

Bacteria and chemicals: The chemicals and bacteria used: Silver nitrate and Muller Hinton agar received from Sigma Aldrich, Luria broth agar, Luria broth, Agar-agar, Peptone, Yeast extract, Dextrose, Magnesium sulphate, Calcium chloride, Potassium dihydrogen phosphate and Disodium hydrogen phosphate received from Himedia. Bacteria used are as follows *E.coli, B.subtilis, Salmonella Sp., Psedomones aerogenes, Vibro-parahaemolyticus* and *Pencillin chrysogenum.*are collected from Life teck Chennai.

A. **Preparation of silver nitrate aqueous solution, media and culture:** Silver nitrateaqueous solution was prepared in 1mM. Lbmedia- LB broth was prepared. M9media - Peptone - 10 g, Yeast extract - 5 g, Dextrose - 2 ml, Magnesium sulfate- 2 ml and Calcium chloride- 0.1 ml, Potassium dihydrogen phosphate and Disodium hydrogen phosphate for each 100 ml. Both medium prepared for 1000 ml.
B. **Production of silver nanoparticles and Parameters optimization:** The different medium culture was centrifuged, supernatant was collected. 5 ml of the

supernatant of each medium was added to $AgNo_3$ aqueous solution separately. The solutions was exposed to sunlight until the colour change takes place. For optimization: Time variation such as 2, 6, 10, 14, 18, 22, 26, 30, 60 and 120 minutes. Concentration variation of silver nitrate such as0.5, 1, 2, 4, 6, 8 and 10 mM. pH maintained at 4, 5, 6, 7, 8 and 9, respectively.

C. **Characterization of nanoparticles:** The synthesized nanoparticles was characterized by UV-vis spectrophotometer (SYSTRONICS DOUBLE BEAM UV-VIS Spectrophotometer: 2202) for the initial conformation of AgNPs. Furthermore, SEM(Carl ZeissMA15 / EVO 18) analysis was done to study its size and morphology, XRD(model Bruker D8 advance PXRD) to study its nature, FTIR (Nicolet Impact 400) and EDAX(Oxford Instruments Nano Analysis) was done to find the element and other components present in the sample.

D. **Antimicrobial assay:** Direct method - In Mueller Hinton agar plate the antibacterial assay was done against *E.coli, B.subtilis, Salmonella sp., Psedomones aerogenes, Vibro-parahaemolyticus.* The antifungal assay was done against *Pencillin chrysogenum* by using potato dextrose agar.The sample was loaded into the well of each plate. After 24 hours zone of inhibition was measured.

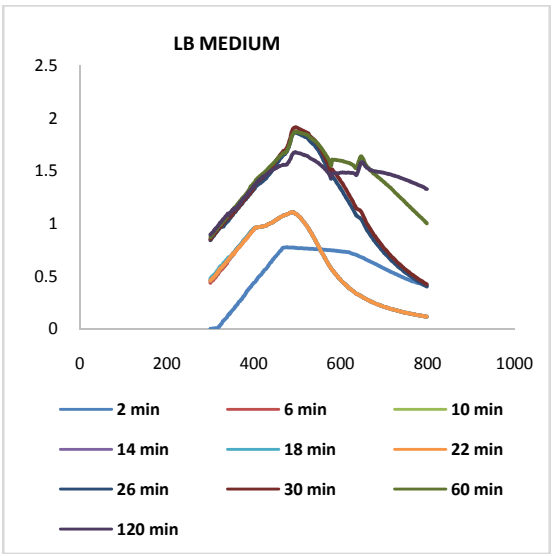
RESULTS AND DISCUSSION

The *E.coli* culture was grown in medium, supernatants was subjected to produce AgNPs by reducing $AgNo_3$ in the presence of sunlight. The colour changed from yellowish to the brown colour solution this shows the formation of silver nanoparticles and the surface plasmon resonance absorption was observed between the range of 400-450 nm (Lihong Liu *et al*., 2014). The parameters such as time, concentration of $AgNo_3$ and pH was optimized by using UV-vis spectroscopy study.

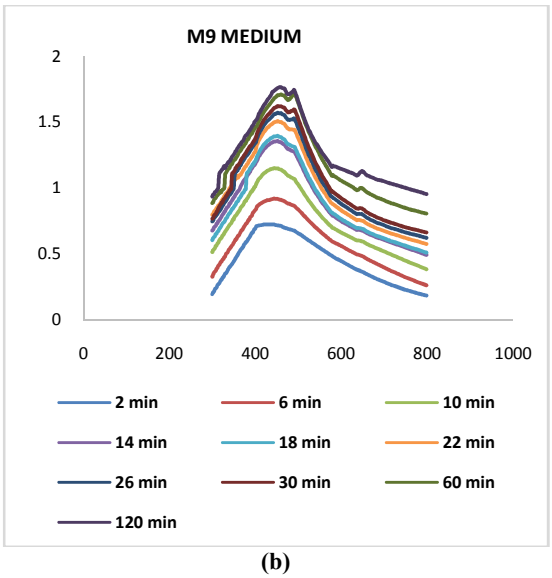
A. UV-vis spectroscopy analysis: Graph recorded from UV-vis spectroscopy between 300-800 nm: Time optimization (Fig. 2, (a) & (b)): LB, a broad peak was obtained from 440-495 nm for 2, 6, 10, 14, 18, 22, 26, 30, 60 and 120 mins respectively. M9 absorbance a broad peak was obtained from 420-444 nm for 2, 6, 10, 14, 18 and 22 mins. Further the peak get shifted to 452 and 456 for 26, 30, 60 and 120 mins respectively (Hossein Ghaforyan *et al*., 2015). Concentration of $AgNo_3$ optimization (Fig. 3, (a) & (b)): LB - was observed from 440-492 nm for 0.5, 1, 2, 4, 6, 8 and 10 mM respectively. M9 - absorbance peak was obtained from396-444 nm and 460 nm for 0.5, 1and 2 mM. Further the peak get shifted to 492 mM for 4, 6, 8 and 10 mM respectively (S. Anil Kumar *et al*., 2006). pH optimization (Fig. 4,(a) & (b)): LB - absorbance peak from 440-492 nm for 4, 5, 6, 7, 8 and 9 respectively. M9 - absorbance peak from 420-444 nm for 7 pH. The peak get shifted to 400 nm for 4 pH and 492 nm for 5, 6, 8 and 9 respectively (Bahareh Khodashenas *et al*., 2005). Hence the peak obtained at 444 nm for M9 but for Lb medium the peak is at 492 nm which shows a negative influence (large particle size) due to the presence of NaCl and nitrate compounds. The other parameters was optimized by UV-vis spectroscopy study, the AgNPs was produced by *E.coli* cultured inM9 medium in a optimum conditions such as 22 minutes in 2mM concentration of silver nitrate at 7 pH.

(a) (b) (c)

Fig. 1 (a) Aqueous solution of AgNo3, Fig (b) Mixture of supernatant and aqueous solution of AgNo3, Fig(c) Formation of AgNPs



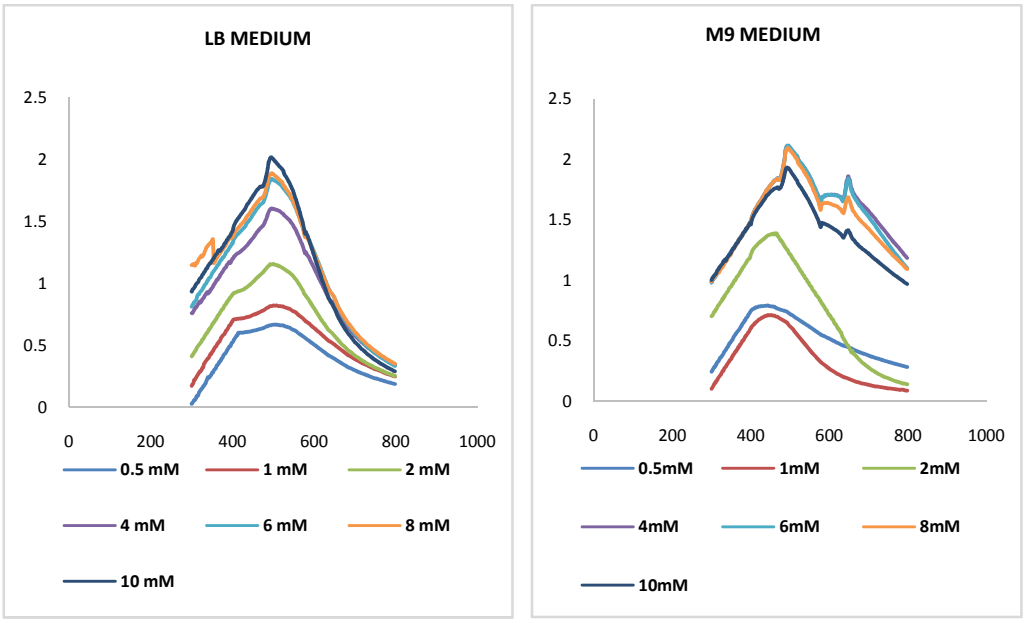
(a)



(b)

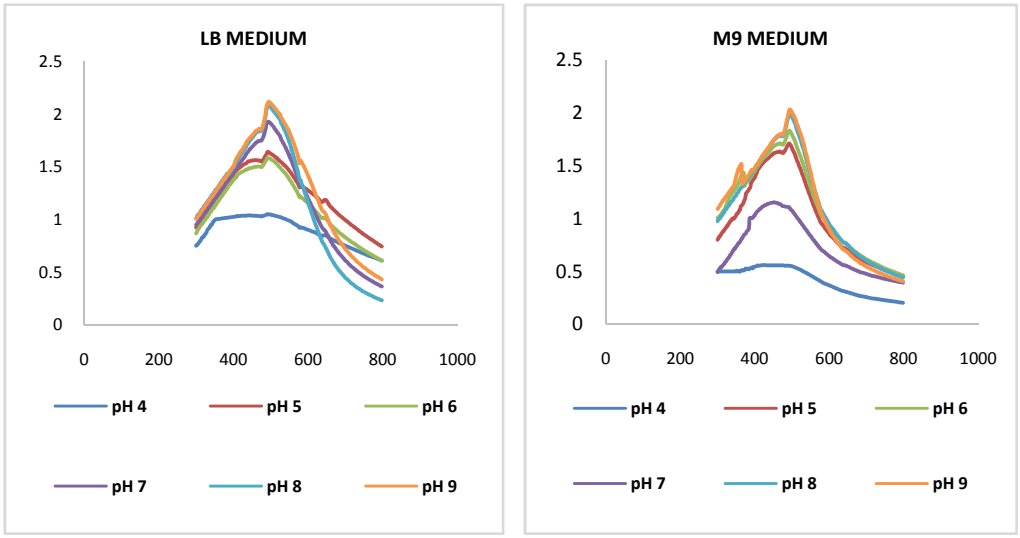
Fig. 2 (a) & (b)UV-vis spectroscopy time optimization

B. EDAX and SEM analysis: The Energy Dispersive spectroscopy study (Figure 5 (a)) shows the presence of elemental silver signal. The Ag nanocrystallites display an optical absorption band peaking at ~3 keV, which is typical of the absorption of metallic Ag nanocrystallites due to the surface plasmon resonance.



(a) (b)

Fig. 3 (a) & (b). UV-vis spectroscopy $AgNo_3$ concentration optimization



(a) (b)

Fig. 4 (a) & (b)UV-vis spectroscopy pH optimization

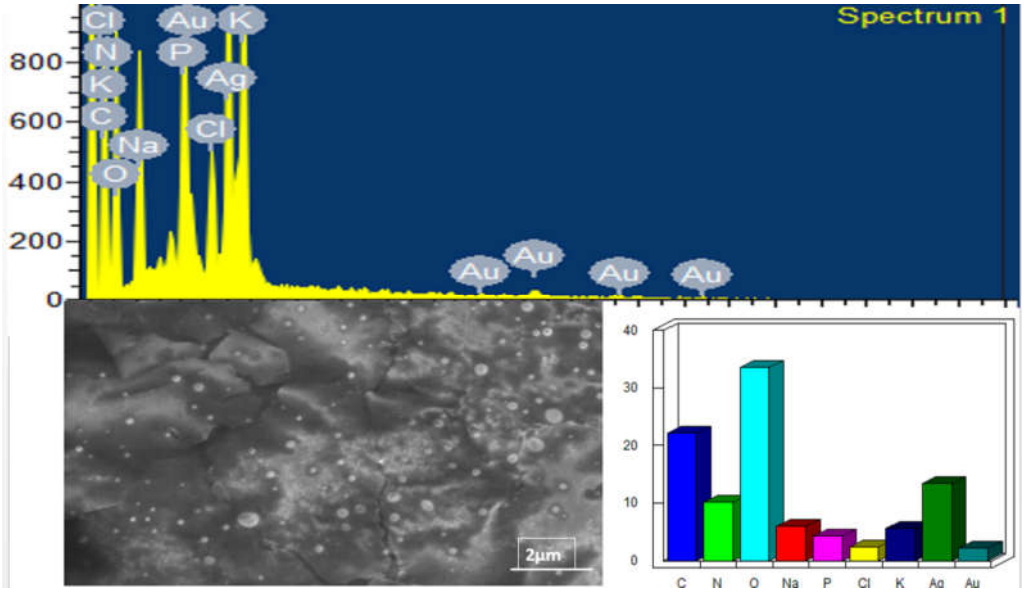


Fig. 5 (a) EDAX of silver nano particle, (b) SEM image of silver nano particles

Table 1. Antimicrobial activity done by on well diffusion assay

Species name	Size of zone in Sample (mm)	Size of zone in control (mm)
B.subtilis	70±20	80±20
Sallmonella Sp	60±20	80±20
Psedomones aerogenes	60±20	80±20
E.coli	60±20	80±20
Vibro-parahaemolyticus	70±20	80±20
Pencillin chrysogenum	80±20	80±20

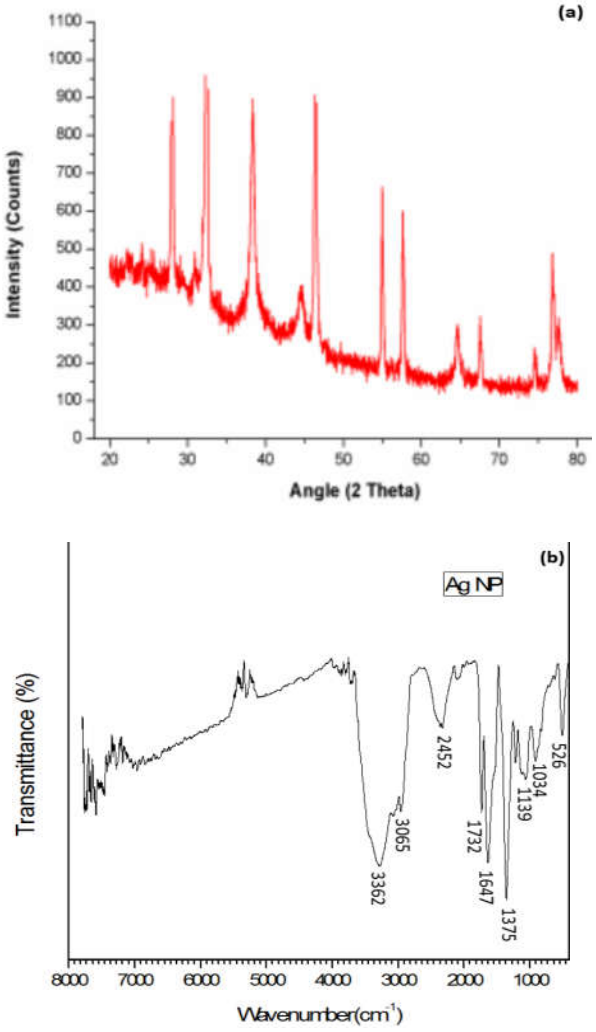


Fig. 6(a). XRD study of silver nano particle, (b) FTIR study of silver nano particle

The presence of carbon indicates the presence of stabilizers. There are many other elements present- N, O, Na, P, Cl and K. The presence of Au is because of the grid is coated with Au. The SEM study (Fig. 5 (b)) shows the range of the AgNPs size is around 65nm were observed. The morphological structure was found to be spheroidal and monodispersed. The particles are not aggregated, which is an indication to the presence of acapping agent.

C. XRD analysis: The X-Ray crystallography study (Figure 6 (a)): The diffracted intensities were recorded from 20 ° to 80 ° at 2θ angles.The spectra of the AgNPs sample exhibits peaks at different θs (28°, 32.4°, 38.3°, 46.4°, 54.9°, 57.6°, 65.2° and 76.8°). The intensity of the peaks indicates the crystalline nature of silver nanoparticles in the sample. The XRD spectrum of the silver particles formed was confirmed in our experiments were in the form of nano crystals (Karthik *et al*., 2016).

D. FTIR analysis: The FTIR spectrum (Figure 6 (b)) reveals two bands at 1732 and 1647 cm^{-1} that corresponds to bending vibrations of the amide I, II bands of the proteins respectively. Corresponding stretching vibrations seen at 3362 $cm^{-1}$and 2452 cm^{-1} shows the presence of protein over layer. The two bands observed around 1375 cm^{-1} and 1647 cm^{-1} can be assigned to the C–N stretching vibrations of the aromatic and aliphatic amines, respectively. Results indicated that the proteins could have also formed a layer along with other bio-organic molecules, which would have played important role in reducing Ag^{+} ions to AgNPs. Secured nanoparticles from aggregation (Probin Phanjom*et al*., 2005).

Conclusion

In this work, we inferred that M9 medium was best for the synthesis of AgNps in comparison with Lb media. The parameters that influences the synthesis of AgNPs such as time, concentration of silver nitrate and pH were optimized by UV-vis spectroscopy analysis. The size of synthesized AgNps was found to be 65 nm (approx) by SEM. The presence of capping agent and Ag element is confirmed by EDAX and FTIR. The synthesized AgNPs was found to be crystalline in nature and shows better antimicrobial activity. This synthesized AgNPs can also be used for various applications such as electrical conductivity, bio-sensors, biological tags for quantitative detection, wound dressings and cosmetics.

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