International Journal of Latest Trends in Engineering and Technology
Special Issue - International Conference on Nanotechnology: The Fruition of Science-2017, *pp.001-004*
e-ISSN:2278-621X, p-ISSN: 2319-3778

ANTIMICROBIAL ACTIVITY OF SILVERNANOPARTICLES PRODUCED BYE.COLI AN ISOLATE OF COIR INDUSTRY

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Abstract-Nanotechnology is gaining tremendous importance in the present century due to its capability of modulating metalsinto their nanosize, which drastically changes the chemical, physical and optical properties of metals. Metallic silver in the form of silver nanoparticles has made a remarkable comeback as a potent antimicrobial agent. The use of silver Nanoparticles is also important, as several pathogenic bacteria have developed resistance against various antibiotics. Hence, in the present investigation the assay was carried out to scrutinize the silver nanoparticle producing microorganism from cashew nut industry, coir industry and cement industry which was further confirmed by biochemical characterization. UV- Visible spectroscopy revealed the first indication of formation of silver nanoparticles from E.coli an isolate from coir industry. Antibacterial activity of synthesized silver nanoparticles was performed against some human pathogens. The observed results revealed that silver nanoparticles were acting against the human pathogens. Maximum inhibitory activity was obtained with the Pseudomonas syringae(24 mm) andStreptococcus pneumonia (17 mm) respectively.
Keywords:Silver nanoparticles, E.coli, human pathogens

Introduction

Nanotechnology involving synthesis and applications of nanomaterials is a rapidly growing field with significant applications in various areas (Duran *et al*., 2005). The attraction of silver nanoparticles (SNPs) is mainly because of its application in therapeutics, biomolecular detection, catalysis and also as antimicrobial agents (Sadhasivam*et al*., 2010; Shrivastava*et al*., 2009; Wei *et al*., 2008; Christopher *et al*., 2011). Traditional chemical methods of synthesizing silver nanoparticles include the use of ethylene glycol, pyridine and sodium borohydride (Geoprincy*et al*., 2014). The chemicals used in these methodologies can be toxic and highly reactive posing a risk to the environment and humans, or the procedures are too expensive to be feasible at an industrial scale. Hence, there is an ever-growing need to develop inexpensive, clean, nontoxic, and environmentally benign synthesis procedures. Consequently, in last years, researchers in nanoparticle synthesis have turned to biological systems for inspiration (Agarwal *et al*., 2014). Microorganisms have recently been found as possible eco-friendly manufacturers, even though they have many biotechnological applications such as remediation of toxic metals (Shen*yet al*., 2011**)**.In this concern, the present study was dealingwith soil microorganisms from industrialized area, which were screened for its potential to synthesis SNPs. The selected strain was subjected to identification by biochemical characterization which resulted in its identification as*E.coli* .The reduction of silver ions was checked by visual inspection as well as by measuring its UV-Visible absorption. Antibacterial activity of synthesized silver nanoparticles was performed against some human pathogens.

Materials and Methods

Collection of soil samples

Soil samples from the surroundings of three different industries of Southern Tamil Nadu such as Cashew nut industry(CI), Coir Industry (CC) and Cement Industry (CM) were collected at a depth of 15 cm and a distance of 1 km away from the industrial sites in sterile conical flasks (Saxena, 1998).

Isolation of Bacteria

One gram soil sample extracted with 100 ml distilled water was serially diluted to 10^{-6} and used as a source of inoculum for the isolation of SNPs producing bacteria. 100 µl inoculum was then plated in nutrient agar and

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incubated at 37°C for 24 hrs. The pure cultures made by streak plate technique were then subjected to screening for silver nanoparticle production (Cappuccino & Sherman, 2013**).**

Synthesis of silver nanoparticles

For the biosynthesis of SNPs, 10 ml of supernatant (morphologically different isolates from the three different soil samples) was mixed with 5ml silver nitrate (SNPs) solution (10 mM) and another reaction mixture without silver nitrate was used as a control. The prepared solutions were incubated at 30°C for 24 h. All solutions were kept in dark to avoid any photochemical reactions during the experiment. After 24 hrs as the solution turned into brown colour from yellow .The SNPs were purified by centrifugation at 10,000 rpm for 5min twice, and collected for further characterization(Durun*et al*., 2005).

Characterization of silver nanoparticles

The optical characteristics of the synthesized SNPs were analyzed using UV–Vis spectrophotometer**(**Muthu & Radhika, 2016). For this, nanoparticle containing samples were subjected to absorption analysis at 200-700 nm range using UV–Vis spectrophotometer (Hitachi U5100).

Antibacterial activity

Antibacterial activity of synthesized SNPs were performed against some human pathogens using disk diffusion method (Cho *et al*., 2005).

Result and Discussion

Morphologically different microorganism was isolated from three industrial sites and it was named as CI1,CI2,CI3,CC1,CC2,CC3,CC4,CM1,CM2,CM3,CM4 respectively (Table 1).

Table 1 : Bacterial isolates obtained from various industrial sites

S.No.	Soil Samples	Collected Sites	Total No. of colonies Obtained
1.	CI	Cashewnut Industry	3 (CI1-CI3)
2.	CC	Coir Industry	4 (CC1-CC4)
3.	CM	Cement Industry	3 (CM1-CM4)

The confirmation of synthesis of nanoparticlesin the medium was characterized by the changes in color of the reaction mixture from light yellow to brown after 24hrs of incubation. Bacterial isolates CC3, showed positive results as color changed to brown, (Table 2)and the isolate was further confirmed as *E.coli* through biochemical characterization (Table 3).

Table 2 : Assay for silver Nanoparticles synthesis

S.No.	Bacterial Isolates	Colour Indication
1.	CI1	Colourless
2.	CI2	Colourless
3.	CI3	Colourless
4.	CC1	Colourless
5.	CC2	Colourless
6.	CC3	Red Colour
7.	CC4	Colourless
8.	CM1	Colourless
9.	CM2	Colourless
10.	CM3	Colourless

Table 3 : Morphological and biochemical characteristics of PHB producing bacterial isolates

S.No.	Morphological Characterization	Result
1.	Gram Staining Method	Circular, entire,smooth
2.	Colony Morphology	Translucent,Colourless
3.	Biochemical Charaaterization	
4.	Indole Production Test	Positive
5.	Methyl Red Test	Positive
6.	VogesProskauer Test	Negative
7.	Citarte Utilization Test	Negative
8.	Catalase Test	Positive
9.	Oxidase Test	Negative
10.	Urease Test	Negative
11.	Starch hydrolysis test	Negative
12.	Arabinose	Positive
13.	Dextrose	Positive
14.	Fructose	Positive
15.	Galactose	Positive
		E.coli

The corresponding UV-Vis absorption spectra are shown in Figure 1. The control solution (without silver nitrate solution) shows no evidence of absorption in the range 300 to 900 nm. The samples exposed to the silver nitrate solution showed the wide spectrum range around 400 nm. The presence of the broad resonance indicates the aggregation of the SNPs in the solution.

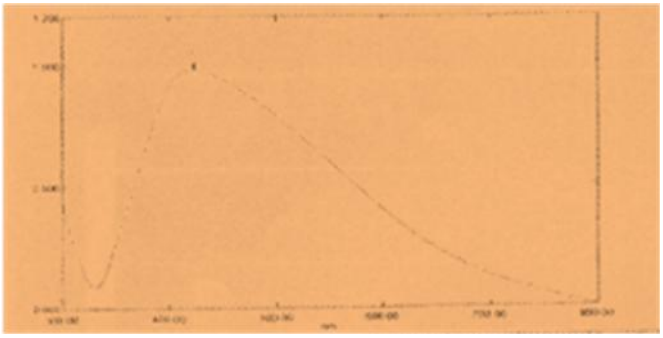

Figure 1: UV- Vis absorption spectra SNPs produced by E.coli

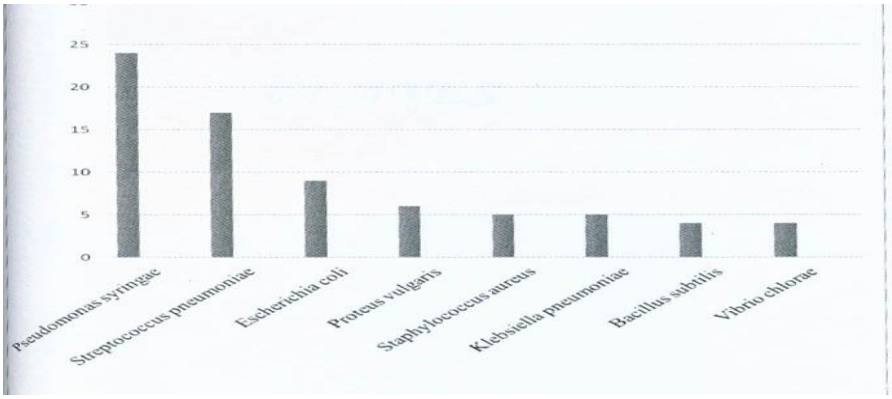

Figure 2 : Antibacterial activity of SNPs produced by E.coli against human pathogens

Antibacterial activity of silver nanoparticles synthesized by *E.coli* against human pathogens revealed that *Psedomonassyringae*produced maximum clear zone of 24mm which was followed by *Streptococcus pnemoniae*with a clear zone of 17 mm. The other microorganismsuch as *Proteus vulgaris,Staphylococcus aureus, Klebsiella*

*pneumonia, Vibrio cholera, Bacillussubtillis*did not show significant activity(Figure 2). According to Lok*et al* . 2006 the attachment of both silver ions and nanoparticles to the cell membrane caused accilimization of envelope protein precursors causing dissipation of the protein motive force. SNPs also exhibited destabilization of the outer membrane and rupture of the plasma membrane, thereby causing depletion of intracellular ATP. According to Lin *et al*. 1998 rupture of bacterial cell membrane may lead to cell death. Siva Kumar *et al*. 2003 proposed that oxygen associated with silver reacts with the sulphydral (S-H) groups on the cell membrane to form R-S-S-R bonds causing inhibition of respiration resulting in cell death.

Conclusion

Biosynthesis of SNPs is an economical, eco-friendly, nontoxic, and simple one step synthesis. The synthesized SNPs were confirmed by the change of colour, UV-Vis absorption of the SPR value at 400 nm.The synthesized SNPs of from *E.coli* has remarkable antibacterial activity against the strains*Psedomonassyringae , Streptococcus pnemoniae* which makes them a potent source of antibacterial agent.

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