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Original Research Article

Biosynthesis of Silver Nanoparticles from Silver Resistance Bacteria Isolated From Metal Contaminated Soil

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Abstract: Nanotechnology involving synthesis and applications of nanomaterials is a rapidly growing field with significant applications in various areas. The attraction of silver nanoparticles (AgNPs) is mainly because of its application in therapeutics, bio molecular detection, catalysis and also antimicrobial agents etc. Microbial synthesis of nanoparticles is eco-friendly and has significant advantages over other processes. In the current study, synthesis of Silver nanoparticles by a bacterial strains isolated from Metal contaminated soil has made. Biosynthesis of silver nanoparticles was confirmed by metal ion reduction colour test which showed colour change from colourless to brown. Molecular identification of nanoparticles producing strains showed the presence of *Bacillus flexus*, *Bacillus pseudomycoides* , *Bacillus flexus* , *Cronobacter universalis*, and *Kocuriarosea*.
Keywords: Biosynthesis, Silver nanoparticles, Metal contaminated soil, *Bacillus flexus*, *Bacillus pseudomycoides* , *Cronobacter universalis*, *Kocuria rosea*.

INTRODUCTION

Nanoparticles are simply defined as particles in the 10^{-9} nm range. Now-a-days we are using nanoproducts in various fields. The nanoparticles possess unique physico-chemical, optical and biological properties which can be manipulated suitably for desired applications [4].

Silver has been used for thousands of years as a precious metal by humans in different applications as jewelry, tools, coins, photographic material or explosives. Zhao and Stevens in 1998 worked on the antimicrobial activity of silver nanoparticles(Ag-NPs),which appears significantly high and also reported that Silver is more toxic element to microorganisms than many other metals in the following sequence: Ag > Hg > Cu > Cd > Cr > Pb > Co > Au > Zn > Fe > Mn > Mo > Sn and Silver exhibits low toxicity to mammalian cells. [11] and [15] investigated that Ag-NPs exert more efficient than silver ions and other silver salts in mediating their antimicrobial activity [16, 10, 18, 7, 20] reported that Silver has a lower propensity to induce microbial resistance than many other antimicrobial materials.

Biological methods can be used to synthesize silver nanoparticles without the use of any harsh, toxic and expensive chemical substances [1-3, 5, 14, 17]. It was reported that highly stable silver nanoparticles (40 nm) could be synthesized by bioreduction of aqueous silver ions with a culture supernatant of some non pathogenic and pathogenic Bacteria viz., *Bacillus licheniformis* [6], *B. subtilis* [23], *Pseudomonas stutzeri* AG259 [9], *Klebsilla pneumonia* [12], *E.coli*, *Enterobacter cloacae*[16] and *Lactobacillus* [13].

It is reported that some bacteria contain plasmid with *Sil* genes which are responsible for silver resistance of bacteria [28]. The mechanism involved in the resistance are efflux system, alteration of solubility and toxicity via reduction or oxidation, biosorption, bioaccumulation, extracellular complex formation or precipitation of metals, and lack of specific metal transport systems [26]. In *invitro* synthesis of silver nanoparticles using bacteria, the presence of NADPH dependent nitrate reductase convert nitrate to nitrite and the electron transferred to silver ion hence the silver ion reduced to silver(Ag+ to Ag0) [27].

Many studies have enlightened the biological synthesis of Silver nanoparticles from bacteria; however, biological synthesis of silver nanoparticles from Silver resistance bacteria isolated from metal contaminated soil is scantily studied. Hence, the present study was carried out to screen a variety of metal resistant bacteria for their ability to produce Silver nanoparticles.

MATERIAL AND METHOD

Isolation of Metal resistant bacteria from soil

Metal contaminated soil samples were collected from different locations viz. jewelry processing shops, silver industries, and silver plating industries etc; were mixed together and considered as Source material for Bacterial isolation. The isolation of metal (Ag) resistant bacteria was done by Replica plate technique using Nutrient Agar Supplemented with 1mM Concentration of filtered sterilized $AgNO_3$ and incubated at 37ºC for 48hrs the plates were observed for the presence of bacterial growth [25]and the cultured isolates were consider as silver resistance bacteria.

Conventional Identification Metal resistant bacteria

The metal (Ag) resistant bacterial isolates were further identified by conventional method [19]. The primary identification of bacterial isolates was done on the basis of colony, microscopic and biochemical characteristics followed by comparing with available standard literature.

Screening for Silver Nanoparticles synthesis

All the metal (Ag) resistant Bacterial isolates were further studied qualitatively for Silver nanoparticles synthesis, adopting method suggested by [8] and [25]. The cultures were cultivated separately at Ph 6 and 40º C Temperature for 24 hours in LB broth. The bacterial cells were separated by centrifugation at 20,000 rpm for 10minutes. The cell free supernatant (filtrate) material was separated out and use for extracellular synthesis of nanoparticles. For nanoparticles synthesis, approximately 0.2 ml of supernatant from each cell free culture was mixed separately with 10ml of 1mM aqueous solutions of filtered sterilized $AgNO_3$, in 20ml test tube and placed in 150 rpmincubatory shaker (Remi make) at 37ºC upto 72 hrs. Qualitative screening for the presence of Silver Nanoparticles was done by periodic Visual observation to check the color change. Appearance of brown color solution indicates the formation of silver nanoparticles in the reaction mixture [22]. The set without $AgNO_3$, was maintained as Control. The isolated bacterial strains positive for Silver nanoparticles synthesis were identified at Molecular level.

Molecular Identification of AgNPs synthesizing bacterial isolates

All the cultures were processed for the Molecular identification by 16S ribosomal RNA gene partial sequence based method. The sequence data were subjected to BLAST and Phylogenetic analysis at MACS Department of Science and Technology, *Govt.* of India, Pune.

RESULT AND DISCUSSION

Isolation of Metal resistant bacteria from soil

It was observed that among the cultured colonies, only 09 isolates were able to grow on nutrient agar supplemented with 1mM concentration of $AgNO_3$;and Hence, considered as Silver resistant bacterial isolates and perhaps be explore for the synthesis of AgNPs. Findings on the existence of silver resistance bacteria in silver contaminated soils are in accordance of [25] they isolated the silver resistant bacteria from heavy metal contaminated areas of Kerala.

The Silver resistant soil isolates showing the growth in presence of $AgNo_3$ were further identified by conventional method; the results obtained were compared with standard literatures and indicated the presence of different morphological forms, cocci and bacilli with variety of arrangements. Total (03) Species of Grams positive bacilli, (01) species of Grams positive cocci and (05) species of Grams negative bacilli were observed. The findings suggest the possible presence of *Bacillus, Enterobacter, Klebsiella, Escherichia Proteus, Cronobacter and Kocuria* species respectively.

Screening for Silver Nanoparticles synthesis

Cell free supernatants of all Silver resistant bacterial isolates were separately treated with 1mM aqueous solutions of filtered sterilized $AgNO_3$. After incubation time the solution were observed for colour change from colourless to brown. The results on qualitative test Table 1 and Photoplate 1, Indicated that among all the isolates, only 5 isolates out of 9 showed the synthesis of AgNp. The isolate number 1,2,4,8 and 9 showed the synthesis of AgNPs within 72 hours, whereas 3, 5, 6 and 7 didn't showed AgNPs synthesis though these isolates showed metal resistance attribute. The findings on the present study are in correlation with the experimental findings of [24] and [21].They reported the colour change due to the biosynthesis of AgNPs.

From the results on the Qualitative screening of AgNPs producer it was also sensible that, the character to have silver resistant trait among the strains must not be taken as the indicator for the synthesis of silver nano particles. The qualitative screened isolates were rebelled as AK 1, AK 2, AK 3, AK 4 and AK5, as AgNPs producers and subjected for Molecular identification.

Table 1: Qualitative screening of Bacterial isolates Synthesizing AgNPs

Isolates No.	Colour Change from Colourless to Brown	Synthesis of AgNPs
1	**Positive**	**+ve**
2	**Positive**	**+ ve**
3	Negative	- ve
4	**Positive**	**+ ve**
5	Negative	- ve
6	Negative	- ve
7	Negative	- ve
8	**Positive**	**+ ve**
9	**Positive**	**+ ve**

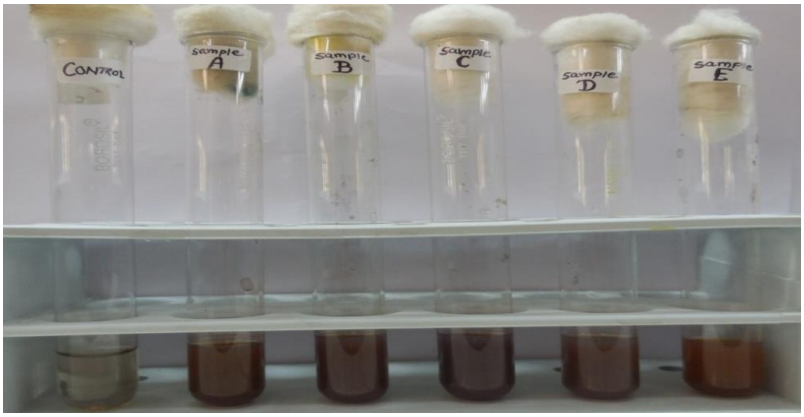

Fig-1: Colour reduction test for Synthesis of Silver nanoparticles

Molecular Identification of AgNPs synthesizing bacterial isolates

Isolate AK1 showed its maximum identity of 100 % *to Bacillus* sp. mainly *Bacillus flexus*. Isolate AK2 showed its maximum identity of 100% to *Bacillus pseudomycoides* . Isolate AK3 showed its maximum identity of 100 % to Bacillus sp. mainly Bacillus flexus. Isolate AK4 showed its maximum identity of 99.2% *Cronobacteruniversalis*. Isolate AK5 showed its maximum identity of 100% *Kocuriarosea* . Phylogenetic analysis of all isolates were represented in Figure 2.

From molecular identification by 16S rRNA sequencing-based method the selected isolates were reported as of AK1- *Bacillus flexus*, AK2- *Bacillus pseudomycoides* , AK3- *Bacillus flexus* , AK4- *Cronobacteruniversalis*, and AK5-*Kocuriarosea.*

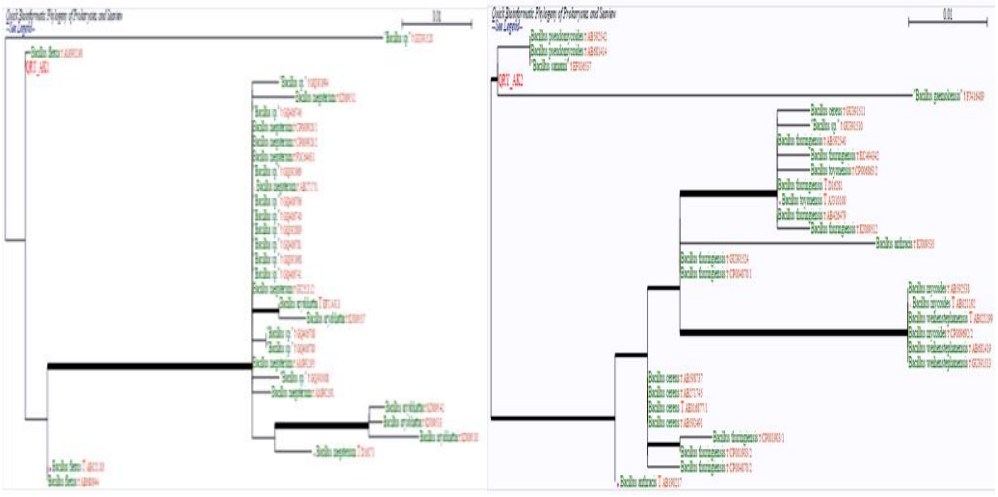

Fig-2: Phylogenetic analysis of the 16S rRNA sequence of the soil bacterial isolates obtained in the study

CONCLUSION

This work demonstrates the ability of bacteria to synthesize the silver nanoparticles and hence can be considered as source of AgNp. The identified isolates viz. *Bacillus flexus*, *Bacillus pseudomycoides*, *Bacillus flexus*, *Cronobacter universalis* and *Kocuriarosea* found to have the potential to form Silver nanoparticles extracellularly.

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