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Biosynthesis of Silver Nanoparticles by Selective Strains and Evaluating Antimicrobial Activity

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The present study deals with an ecofriendly method for synthesising Silver Nano Particles (SNPs) from the supernatant of three selective test strains namely *Escherichia coli, Bacillus* sp and *Lactobacillus* sp. The synthesis of SNPs were confirmed by UV-VIS spectroscopy which revealed Surface Plasmon Resonance (SPR) absorption band located at 400nm for *Bacillus* sp and 420nm for both *E.coli* and *Lactobacillus* sp. The antibacterial properties of SNPs were evaluated against Gram positive *Staphylococcus aureus* and Gram negative *Pseudomonas aeruginosa* with control plates. The SNPs of *Bacillus* at 5µl and 10µl concentration showed considerable inhibitory zone against *P.aeruginosa* and *Staph. aureus* followed by SNPs of *E.coli* and *Lactobacillus* sp. The application studies also carried out to emphasize the bactericidal activities of SNPs on cotton textiles towards Gram negative organisms *E.coli* and *P.aeruginosa* and they revealed zone of clearance.

Key words: SNP, SPR, UV-VIS Spectrum, MHA plates, Zone of inhibition.

Nanoparticles exhibit completely new and improved properties based on specific characters such as size, distribution and morphology, if compared with larger particles of the bulk[1]. Currently nano sized organic and inorganic particles find increasing applications in medical devices as the result of their amenability to biological functionalization [2]. In recent years, the development of microbial sources has a potential effect on the synthesis of metallic nanoparticles such as silver, cadmium, sulphide, gold, tin and nickel[3]. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required than other techniques[4-6]. The main drawback of using chemical reduction in nanoparticle synthesis is most of the reactants used in the reaction system is toxic chemical agents which have potential risk for environment and health. Biological reduction is recently developed as a promising method because of its greater advantage such as sufficient material sources, mild reaction conditions, good dispersion of nanoparticles and microorganisms as possible ecofriendly nanofactoreies[7] .

Silver is a health additive in traditional Chinese and Indian ayurvedic medicine[8]. Silver could be used for the treatment of infections in burns, open wounds and chronic ulcers and these are the beneficial as diabetic wound healing agents as they are mainly affected by many secondary infections[9]. Silver has long been recognized as having inhibitory effects towards many bacterial strains and microorganisms commonly present in medical, industrial process[10]. It is non toxic, safe

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inorganic antibacterial agent used for centuries and is capable of killing about 650 types of diseases causing microorganisms[11] including anti-inflammatory actions even at minute concentrations.

In microbes the silver ions strongly interact with thiol groups of vital enzymes and inactivate them. The DNA loses its replication ability once the bacterium treated with silver ions[12].Silver nanoparticles destabilize plasma membrane potential and cause depletion of levels of intracellular adenosine triphosphate (ATP) by targeting bacterial membrane resulting in bacterial cell death[13]. So the present study has been aimed to study the biosynthesis of SNPs from *E.coli*, *Lactobacillus* sp and *Bacillus* sp and their antibacterial properties were evaluated against Gram positive *Staphylococcus aureus* and Gram negative *Pseudomonas aeruginosa* with control plates. The application studies also carried out to demonstrate the bactericidal activity of SNPs on cotton textiles towards Gram negative organisms *E.coli* and *P. aeruginosa.*

MATERIALS AND METHODS

Collection of sample

In this present study, milk sample was collected from milk yard at Kovilpatti, and sewage sample was collected from sewage water treatment plant of National Engineering College, Kovilpatti. Sterile conical flasks were used to collect the samples without any contamination and these samples were subjected to further analysis.

Isolation, characterization and Identification of bacterial culture

The bacterial cultures were screened from the samples by inoculating them individually in 50 ml sterile MRS broth and Nutrient broth medium in 250 ml Erlenmeyer flasks at 37^0C for 24-48 hours. Then these cultures were streaked on sterile selective media like MRS agar for *Lactobacillus* sp, EMB plates for *E.coli*, and Bacillus isolation agar for *Bacillus* sp.

Thus obtained colonies were stored in agar slants at refrigiration condition and further used for microbiological and biochemical characterization studies. Cultures obtained were periodically checked for free of contamination by streak plating and sub culturing. Their confirmation was based on their morphological, physiological and biochemical characteristics upto generic level adopting the schme recommended by Aneja (1996) [14] (table 1).

Production of biomass

Microbiologically and biochemically confirmed *E.coli, Lactobacillus* sp and *Bacillus* sp were cultured to produce biomass for biosynthesis of nanoparticles in nutrient and MRS broth individually. The inoculated culture flasks were incubated on the shaker at 37^0C for 24-48 hours. Then the biomass were harvested and centrifuged at 12,000rpm for 10 minutes followed by filtration through whatman no:1 filter paper in a sterile condition. Thus the collected cell filtrate or extract was further used for synthesis of nanoparticles.

Biosynthesis and Characterization of silver Nanoparticles

Equal volume of 20 ml supernatent at 1mM concentration was separately added to a reaction vessel containing 20ml of different supernatants of appropriate strains. The reaction was carried out under bright conditions for about 48 hours and a control (without adding the silver nitrate) was run along with experimental conditions.

The synthesized silver nanoparticles were measured by visual inspection of the culture vessel for a change in colour of culture medium from a clear, light yellow to yellowish brown. The reduction of silver ions from silver nitrate to silver nanoparticles were confirmed by recording the UV-VIS spectrum of reaction medium at 380-420nm.

Antibacterial activity

The antibacterial activities of synthesized silver nanoparticles were tested by well diffusion method. Sterile Muller Hilton agar plates were prepared and the cultures *Pseudomonas aeroginosa and Staphyloccus aureus* were swabbed on these plates. The silver nanoparticles was added to the wells and kept for incubation at 37^0C for 24 hours. After the diameters of inhibition zones were measured to estimate its inhibitory effects.

Application of Antibacterial Activity on Textile

Cotton fabrics were used for application study purpose. The cotton fabric (4×4cm) was sterilized and immersed into the nanoparticle solutions individually followed by drying for 15 minutes aseptically.

The antibacterial activities of nanoparticles were evaluated against *E.coli* and *Pseudomonas aeroginosa.* Fabric samples were placed on the centre of agar surface where it has been already swabbed with these test organisms. After incubation for about 24 hours, the plates were observed for the zone of inhibition around the fabric sample and the zone of clearance was measured.

RESULTS

Isolation and identification of bacterial strains

The samples collected from different sources were processed to isolate *Lactobacillus* sp, *Bacillus* sp and *E.coli.* Their confirmation was performed and tabulated **(**Table 1).

Biosynthesis of SNP and UV-VIS Spectral analysis

The reduction of $AgNO_3$ was carried out with the supernatant of overnight grown microbial culture. The colour changes of the cell free extract with 1mM $AgNO_3$ happened from pale yellow to yellowish brown in 48 hours. Control without $AgNO_3$ showed no colour change in the cell filtrate when incubated under same condition.

The presence of silver nanoparticles was confirmed by UV-Visible Spectroscopy. This clearly indicated that the reduction of the ions occurred extracellularly through reducing agents released into the solution. Surface Plasmon Resonance (SPR) absorption band is located at 400nm for *Bacillus* sp and 420nm for both *E.coli* and *Lactobacillus* sp (Fig.1)

E.coli synthesized SNP, 2- *Bacillus* synthesized SNP, and 3- *Lactobacillus synthesized SNP)*

Antibacterial activity

The present investigation indicated that the nanoparticles synthesized from *E.coli*, *Bacillus* sp and *Lactobacillus* sp (Table 2) had antibacterial activity aganist Gram negative *Pseudomonas aeroginosa and* Gram positive *Staphylococcus aureus* at 5µl and 10µl concentrations. The inhibition zone was compared with the standard antibiotic tetracycline.

Coating of cotton fabric with nanoparticles and assessment of antibacterial activity

The plates were observed for zone of inhibition around the silver nanoparticles dipped sample and the zone of clearance was measured in millimetre. The effect of antimicrobial activity was tested against Gram negative organism *E.coli* and *Pseudomonas aeroginosa (*Table 3). The high inhibition level was observed in *E.coli* (19mm) by

Table 1. Biochemical analysis of selected isolates

Biochemical test	*Bacillus sp*	*E.coli*	*Lactobacillus sp*
Staining			
Simple staining	Rod	Rod	Rod
Grams staining	+	-	+
Spore staining	+	-	-
Motility	+	+	-
Hydrolysis			
Starch	+	-	-
Lipid	-	+	-
Casein	-	-	-
Catalase test	+	+	-
Triple sugar iron test	-	-	-
Test for carbohydrate fermentation			
Dextrose	+	+	+
Mannitol	+	-	-
Sucrose	+	-	+
IMViC test			
Indole	-	-	-
MR reaction	+	+	-
VP reaction	+	-	-
Citrate utilization	+	-	-

Table 2. Antibacterial effect of SNPs

SNP producing organisms	Zone of inhibition at 5µl and 10µl con/disc(mm) (n=4) Test pathogens				Control Tetra cycline	
	P.aeruginosa 5µl con/disc	*Staph.aureus* 10µl con/disc	*P.aeruginosa* 5µl con/disc	*Staph.aureus* 10µl con/disc	(µg/disc)	
Bacillus sp	3.9 ± 0.82	7 ± 0.21	5.6 ± 0.10	9.6 ± 0.09	9 ± 0.76	12 ± 0.20
E.coli	2.6 ± 0.08	5 ± 0.07	4.0 ± 0.71	6 ±0.12		
Lactobacillus sp	2.1 ± 0.44	4 ± 0.08	3.0 ± 0.70	5.5 ± 0.20		

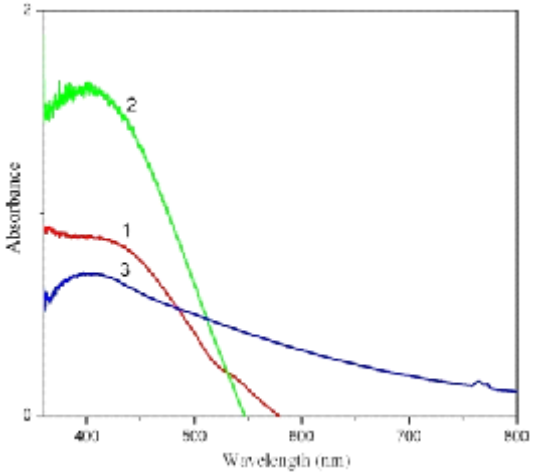

Fig. 1. UV-spectral analysis of biosynthesized SNP

Bacillus synthesized SNP, followed by *E.coli* synthesized SNP(18mm) and *Lactobacillus* synthesized SNP (16mm). *Pseudomonas aeroginosa* showed the inhibitory level (9mm) in *Bacillus* synthesized SNP followed by *E.coli* synthesized SNP (7mm) and *Lactobacillus* synthesized SNP (6mm).

DISCUSSION

It is possible to reduce silver nitrate into SNP enzymatically by microorganisms. Many effective strains like *Bacillus megaterium, B.licheniformis, Enterobacter, Klebsiella pneumonia, Aspergillus* etc[15-16] used for the reduction of silver nitrate either individually or in combinations. The appearance of yellowish brown colour in the reaction is due to the formation of SNPs during the period of incubation[17-18]. The appearance of brown colour in the medium could be due to the excitation of SPR[19]. In the present study also the colour change of supernatent coincides with the earlier findings.

Nowadays different instrumental studies are available for the diagnosis of SNPs production from different types of sample. UV-VIS spectroscopy could be an interesting alternative to consider not only for the identification of nanoparticles production but also to know the characteristics of the isolates. The major advantages of UV-VIS spectroscopy are its rapidity, sensitivity and the possibility to detect the production of nanoparticles from any type of

Table 3. Bactericidal effect of SNPs on cotton fabric (4x4cm)

Test pathogens	Zone of clearance (mm) (n=4)		
	SNPs of *Bacillus* sp	SNPs of *E.coli*	SNPs of *Lacto bacillus* sp
E.coli	19.1 ± 0.21	18 ± 0.70	16 ± 0.54
P. aeruginosa	9.0 ± 0.60	7.0 ± 0.46	6.0 ± 0.43

microorganisms. Similar type of studies by using UV-VIS spectrosacopy have also been applied for the detection of nanoparticles from other organisms such as *Coriolus versicolor, Klebsiella pneumonia,E.coli,Enterobacter cloacae, Solonum torvum*[20-21]. The SNP synthesised by *Solonum torvum* and *Aspergillus fumigates* had high antimicrobial activityagainst bacterial and fungal pathogens[22-23]. Mostly the SNPs produced are very active against various human pathogenic organisms like *E.coli, Staphylococcus epidermis,Serratia* sp and *Salmonella typhi* [24-25] Similarly in this study also bacterial synthesized nanoparticles which were tested against *Staph.aureus* and *P.aeruginosa* showed considerable zone of inhibition around the well.

In earlier findings the fabrication with silver nanoparticles showed bactericidal effect towards many pathogens such as *Staph.aureus, E.coli, P.aeruginosa* and *Aspergillus*[26]. Similarly in the present investigation, the SNPs coated cotton fabrics showed efficient bactericidal activities towards *E.coli* and *P.aeruginosa*. Comparatively *E.coli* showed high zone of clearance than *P.aeruginosa*. From the results obtained in the present study, it is evident that the biological synthesis of nanoparticles provides a wide range of environmentally acceptable methodology, low cost production and minimum time requirement. Biological reduction provides greater advantages such as sufficient material sources, mild reaction conditions, and good dispersion of nanoparticles. Microorganisms as possible ecofriendly nanofactories and the antibacterial properties of synthesised nanoparticles give the way for various practical applications to eradicate drug resistance pathogens in developing countries reducing the cost of production in pharmaceutical industries.

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