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Synthesis of Metal Oxide Nano Particles by *Streptomyces* Sp for Development of Antimicrobial Textiles

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Abstract: The synthesis of metal oxide nanoparticle has received considerable attention with their potential applications in various field. Recently, there has been tremendous excitement in the study of nanoparticle synthesis by using some natural biological system, which has led the growth of advanced nanomaterials. In the present study, we have demonstrated the synthesis of metal oxide nanoparticles by a *Streptomyces sps* isolated from a site Pichavaram mangrove in India**.** Extracellular production of metal oxide nanoparticles by *Streptomyces* sps was carried out. It was found that copper sulphate and zinc nitrate when exposed to *Streptomyces* sps are reduced in solution, thereby leading to the formation of metal oxide nanoparticle. The metal oxide nanoparticles were in the range of 100-150 nm. The possibility of the reduction of metal ions may be by reductase enzyme. The antibacterial and antifungal activity of nanoparticle-coated fabric was evaluated according to the procedures of AATCC147 method and AATCC 30 method, respectively. A 100% reduction of viable *E. coli, S. aureus and A.niger* was observed in the coated fabric materials after 48 h of incubation. Nanoparticle shows promise when applied as a coating to the surface of protective cloth by reducing the risk of transmission of infectious agents.

Key words: Nanoparticles · *Streptomyces* · Pichavaram mangrove · Antimicrobial

INTRODUCTION

An important area of research in nanotechnology is the synthesis of nanoparticles of different chemical compositions, sizes, shapes and controlled disparities. Currently, there is a growing need to develop environmentally benign nanoparticle synthesis processes that do not use toxic chemicals in the synthesis protocol. As a result, researchers in the field of nanoparticle synthesis and assembly have turned to biological systems for inspiration. Microorganisms play an important role in toxic remediation through reduction. Living organisms have huge potential for the production of nanoparticles or nano devices of wide applications. Exposure of the Actinomycete biomass to aqueous copper ions leads to either intracellular or extracellular reduction of metal ions and formation of nanoparticles depending on actinomycetes used. These nanoparticles exhibit tolerable monodispersity and in the case of particles synthesized extracellularly, exhibit excellent long-term stability. The reduction of metal ions occur through an enzymatic process thus opening up the exciting possibility of extending the actinomycetes based method for the synthesis of nanoparticles. These enzymes are involved in the reduction of the metal salts and capping of these reduced metal and metal oxides at nanoscale.

These particles can be incorporated in several kind of materials such as cloths. These cloths with copper nanoparticles are sterile and can be useful in hospitals to prevent or to minimize infection with pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus*. Enhancement of textile materials by nanotechnology is expected to become a trillion dollar industry in the next decade with tremendous technological, economic and ecologic benefits [1]. Antibacterial agents were used on textiles thousands of years ago, when ancient Egyptians used spices and herbs as preservatives in mummy wraps [2, 3].

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In order to explore the potential of nanoparticle-coated fabrics to protect against infectious agents, a study was undertaken to investigate the antimicrobial effect of: (i) nanoparticles containing a combination of copper sulphate with *Streptomyces* sps and zinc nitrate with *Streptomyces* sps (ii) nanoparticle-coated fabrics.

MATERIALS AND METHODS

Isolation and Screening of Actinomycetes from Sediment Soil: Isolation of actinomycetes were performed by soil dilution plate technique using glycerol yeast extract agar complemented with nystatin (50µg/ml) at 27°C [4]. Sediment soil sample from Pichavaram mangrove ecosystem (Lat, 11°27' N; Long. 79°47' E), situated along the southeast coast of India were collected and processed. Actinomycete isolates were distinguished from other microbial colonies by characteristics such as rough, leathery colonies. Then isolated colonies were preserved in glycerol based media and stored at 20°C.

Antimicrobial activities of pure actinomycete cultures performed by using spectra-plak method. Muller hinton agar plates were prepared and inoculated with actinomycetes cultures by a single streak of inoculum in the center of the petridish and incubated at 27°C for four days. Later the plates were seeded with test organism by a single streak at a 90°C angle to actinomycetes strains. Antagonism was measured by the determination of the inhibition zone [5].

Identification of Potential Actinomycete Isolates: The most potent actinomycete isolate which showed activity against both bacteria and fungus was characterized by macroscopic, microscopic and morphological methods. Morphological observation carried out by the methods and media proposed by Shirling and Gottlieb [6]. The chemical compositions of cell wall and whole-cell hydrolysates were analyzed by the methods of Lechevallier and Lechevalier [7].

Synthesis of Copper and Zinc Nano Particles and Assessment of Antimicrobial Activity: The Erlenmeyer flask with the *Streptomyces* sps biomass and supernatant were a yellow color before the addition of copper and zinc and this changed to a brownish color on completion of the reaction. The appearance of a brown color in solution containing the biomass suggested the formation of copper and zinc nano particles. The solution was extremely stable, with no evidence of flocculation of the particles even several weeks after reaction. Aliquots of the reaction solution were taken after 24, 48 and 72 hrs and the antibacterial activity was observed by well diffusion method.

Coating of Cotton Fabric with Nanoparticles: The fine- medium weight 100% cotton fabric was used for the application purpose. Nanoparticles were applied to the fabric by pad-dry-cure-method. The cotton fabric cut to a size of 30 x 30 cm was immersed in a nanoparticle solution. Then it was passed through a padding mangle. After padding the fabric was air dried and then cured for 3 min at 140° C. The fabric was then immersed for 5 min in sodium lauryl sulphate to remove unbound particles.Then fabric was rinsed to remove soap solution [8].

Assesment of Antibacterial Activity (AATCC147 Method) of Nano Finished Fabric: The antibacterial activity was qualitatively evaluated against *Staphylococcus aureus* (ATCC 6538) an d *Escherichia coli* (ATCC 8739). Fabric samples of 2.1 ± 0.1cm diameters were placed in the centre of the AATCC bacteriostasis agar plate previously swabbed with the bacterial inoculum. After 24 hours of incubation, the plates were observed for the zone of bacteriostasis around the fabric sample and the zone of clearance was measured in millimeter.

Assesment of Antifungal Activity (AATCC 30 Method) of Nano Finished Fabric: Broth culture of *Aspergillus niger* (ATCC 6275) was used as inoculum. Using sterile cotton swab, the test organism was inoculated over the surface of the PDA plate. The treated and untreated fabrics were dipped in the inoculum and placed in the centre of the mat culture. The plates were incubated at room temperature for 48-72 hrs in upright position. The untreated fabric containing plate was maintained as a control.

Evaluation of Pathogenic Microbial Reduction Percentage: Specimens of the test material were shaken in a known concentration of microbial suspension and the reduction in microbial activity in standard time was measured. The efficiency of the antimicrobial treatment is determined by comparing the reduction in microbial concentration of the treated sample with that of control sample expressed as a percentage reduction in standard time. Percentage reduction was calculated using the following formula

$$R = (B-A)x100/B$$

Whereas R is percentage reduction, A is the number of microbe in the broth inoculated with treated test fabric sample after inoculation over 24 hrs contact time. B is the number of microbe recovered from the broth inoculated with untreated control after inoculation at 0 contact time.

Wash Durability of the Finished Fabric: The wash durability testing of the finished fabrics was carried out using a neutral soap at 40°C (± 2°C) for 30 minutes, keeping the material: liquour ratio at 1: 50, followed by rinsing washing and drying. After drying the test fabrics and the control were assessed for antimicrobial activity [9].

Characterization of Nano Finished Fabric by SEM and FTIR: The surface topography of copper nano particles finished fabric was observed with a Scanning Electron Microscope (SEM). The physical properties of the finished fabric were determined and the values compared with those of unfinished fabric which served as the control fabric. The samples were analyzed for their variations in chemical groups using FTIR spectroscopy.The wavelength of light absorbed is characteristic of the chemical bond can be seen in this annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.

RESULT AND DISCUSSION

Isolation and Screening of Antimicrobial Actinomycetes: In the present study, 42 different actinomycetes were isolated from the mangrove sediment sample. The antimicrobial activities of the isolates were varied. It was found that 20 strains suppressed the test microorganisms in different degree. The best actinomycete isolate KUA106 has been selected for further study since it showed the greater activity against *S. aureus E. coli* and *A.niger.*. Mangroves are one of the most valuable biomes providing an economic, ecological, scientific and cultural resource. Mangroves have very specialized adaptations that enable them to live different condition. It exists under very hostile and inhospitable conditions [10].

Characterization of the Most Potent Actinomycete Isolate (KUA106): Macroscopic and microscopic methods were employed to describe the potent strain. According to waksman [4] such color form are exhibited by colonies of *Streptomyces.* (Table 1). However, the color and form of the colony could serve as bases for pointing out the genus to which actinomycete belongs to. Hence, its morphological properties must serve as the primary bases of characterization. After growing on the YMA, it was seen to exhibit a grey colony. The form of the colony was described to be compact raised. It was also observed that it produced a yellow diffusible pigment in the medium.

Hence it is suggested that the strain isolated from Pichavaram mangrove environment, possessing reduction activity to form the nano paticles and could be assigned to *Streptomyces sps* and it is worth investigating actinomycetes future in detail for human welfare.

Assessment of the Antibacterial Activity of Zinc and Copper Nano Particle: After fermentation actinomycete biomass was obtained by filtration. The harvested supernatant and pellet were used for synthesis of nanoparticles. The aliquots of the reaction mixture were taken and antibacterial activities were determined by well diffusion method. (Tabls 2 and 3).

Table 1: Cultural morphological characteristics of potent strain

Cultural/Morphological characteristics/	Observation
Temperature	28°C (Mesophilic)
p H	7.2
Color of the colony	White then Grey
Form of the colony	Compact, folded then raised.
Pigmentation in medium	Yellow diffusible pigment
Substrate mycelium	Present
Aerial mycelium	Present
Melanin pigment	Present
Spore chain	Spirales
Spore surface	Smooth
Diaminopimelic acid (DAP)	LL- DAP
Antibiosis against to	*S. aureus* and *E. coli* and *A.niger*

Table 2: Assessment of the antibacterial activity of biomass and supernatant after addition with Zinc nitrate

	Staphylococcus aureus			*Escherichia coli*		
Antibacterial components	After 24 hrs	After48 hrs	After72 hrs	After 24 hrs	After 48 hrs	After 72 hrs
$ZnNo_3$	17mm	16mm	14mm	15mm	14mm	13mm
Biomass + $ZnNo_3$	24mm	24mm	24mm	22mm	22mm	24mm
Supernatant+ $ZnNo_3$	24mm	24mm	24mm	21mm	23mm	24mm

Table 3: Assessment of the antibacterial activity of Streptomyces biomass and supernatant after addition with copper sulphate

	Staphylococcus aureus			*Escherichia coli*		
Antibacterial components	After 24 hrs	After48 hrs	After72 hrs	After 24 hrs	After 48 hrs	After 72 hrs
$CuSO_4$	19mm	20mm	20mm	15mm	14mm	14mm
Biomass + $CuSO_4$	32mm	41mm	41mm	29mm	35mm	35mm
supernatant+ $CuSO_4$	30mm	39mm	40mm	27mm	30mm	30mm

Table 4: Antibacterial activity of coppernanofinished fabric

Test organism	Pellet+$Cuso_4$	Supernatant+$Cuso_4$
Staphylococcus aureus	43mm	40mm
Escherichia coli	36mm	32mm

Table 5: Antimicrobial activity (Microbial reduction) of cotton fabric containing nanoparticles against microorganism

Test organism	control	Treated test	% of reduction
S.aureus	11×10^7	29×10^2	99
E.coli	11×10^7	21×10^5	80
A.niger	10×10^6	28×10^2	98

From the above results, it is clear that antibacterial activity minimum for zinc nitrate treated supernatant and pellet even after 72 hours of incubation. But, when copper sulphate treated extract checked for the antibacterial activity, there was gradual increase after 24, 48 and 72hrs. After 72hrs of incubation, maximum zone of inhibition was obtained as shown in Table 3. When compared with the antimicrobial activity of both supernatant and pellet containing copper sulphate, pellet containing copper sulphate showed greater activity. So here we concluded biomass containing copper sulphate was reduced as copper oxide nanoparticles extracellularly.

Antibacterial Activity of Nanofinished Fabric (AATCC147): The bacteriostatic activity of the copper impregnated fabrics against *S. aureus and E. coli* were studied and this activity was indicated by zone of inhibition (Table 4). However, the cotton fabric with copper nanoparticles showed maximum antibacterial activity. This result demonstrated that copper nanoparticles can be used to prepare sterile fabrics (Fig. 1).

Antifungal Activity (AATCC 30): The antifungal activity of the copper impregnated fabrics against *Aspergillus niger* was studied and this activity was indicated by zone of mycostasis. The antifungal activity of cotton fabrics with and without copper nanoparticles was evaluated. In the fabrics without copper nanoparticles (control) no zone of mycostasis was observed. However, the cotton fabrics with copper nanoparticles presented maximum zone of mycostasis (Fig. 2).

Natural fibres are more prone to microbial attack due to inherent characteristics such as moisture regain, chemical composition and residues left after chemical processing, which provide room for infestation by microbes [11]. Besides, the climatic conditions also influence the microbial activity, in terms of relative humidity and temperature. Textile materials can be exposed to contamination with microbes during production, usage or Storage. Microbial infestation causes cross infection by pathogens and development of odour where the fabric is worn next to skin [2]. Basically, with a view to protect the wearer and the textile substrate, antimicrobial finish is necessary to textile materials. So far no reports are available about the actinomycetes isolated from mangrove sediments exhibiting prominent nano particle production.

Treated cotton fabric showed very high activity with the reduction of bacteria and fungus. The results of the percentage reduction test correspond with that of well diffusion method. Wash durability test carried out with test fabrics showed that significant antimicrobial activity retained in the copper oxide nano particles treated fabrics up to eight wash. After eight wash percentage of reduction was low. No activity found after seventeen wash.

Topographical Analysis by Scanning Electron Microscope: Biomass treated copper sulphate finished fabric was selected to characterize by scanning electron microscope. The surface of treated cotton fabrics were analysed by scanning electron microscope to observe the size and shape of the nanoparticles. The nanoscaled copper particles were observed on surface of the cotton fabric. The nanoparticles were well dispersed on the fibre surface. Scanning electron microscope analysis was done to measure the size of nanoparticles. In this analysis 100-200 nm sized copper nanoparticles were obtained. The particle size plays a primary role in determining their adhesion to the fibre. It is reasonable to expect that the largest particles agglomerates will be easily remove from the fibre surface, while the small particles will penetrate deeper and adhere strongly into fabric matrix. The SEM analysis of the treated fabrics showed nano particles embedded on to the fabrics.

Fig. 1: Antibacterial activity of Pellet containing $Cuso_4$ finished fabric against staphylococcus aureus and Escherichia coli

Fig. 2: Antibacterial activity of supernatant containing $CuSo_4$ finished fabric against staphylococcus aureus and Escherichia coli

Antifungal activity of biomass containing Copper sulphate

Antifungal activity of supernatant containing Copper sulphate

Fig. 3: Antifungal activity of nano finished fabric

Fig. 4: Antifungal activity of untreated fabric

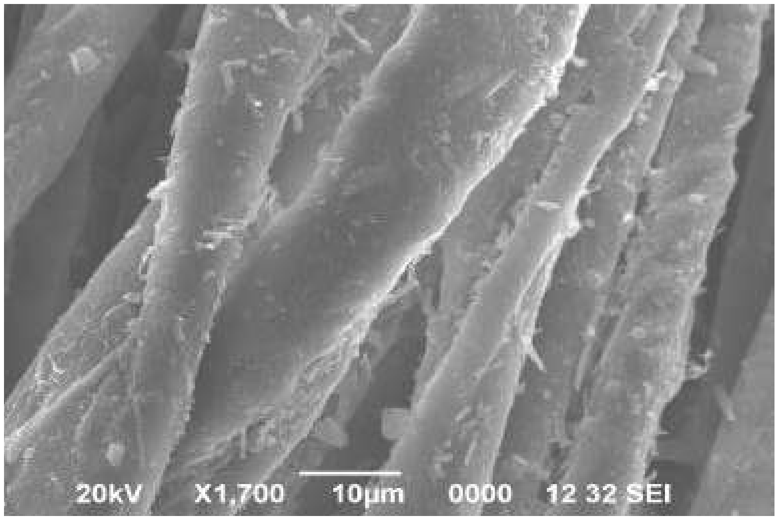

Fig. 5: Scanning electron micrographs of the copper nanofinished cotton fabric, showing nanoscaled copper particles on the cotton fabric

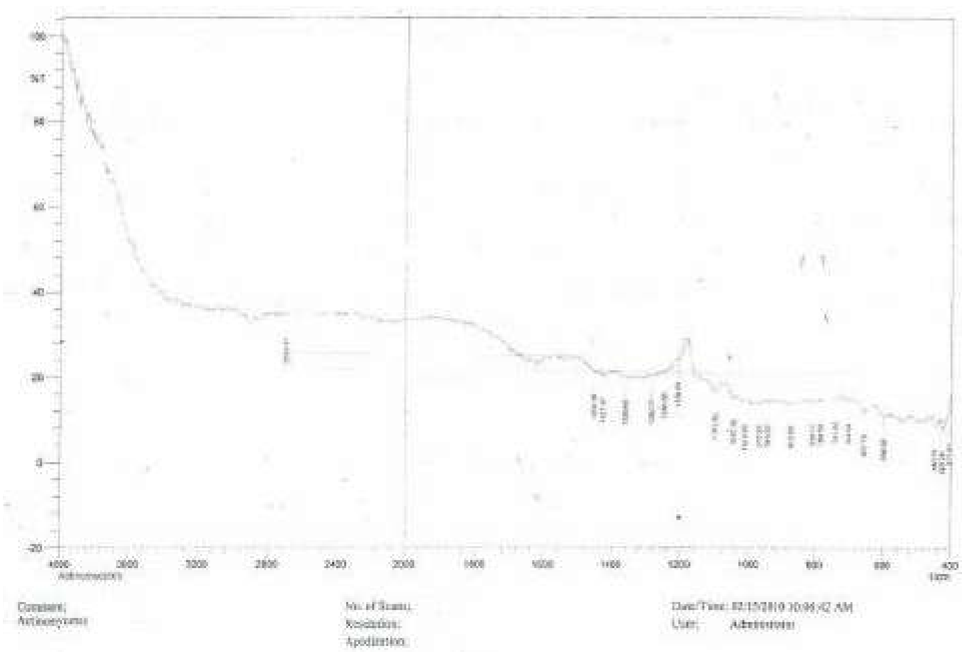

Fig. 6: FTIR analysis of control fabric

FTIR (Fourier Transformed Infrared Spectroscopy): The FTIR analysis was done for copper nanoparticle treated and untreated fabric. When the FTIR spectrum of untreated and treated fabrics were compared, it was found that almost all the absorption peaks were modified upon treatment with copper nanoparticles. Further, it revealed that, the absorption in the region 1000 to1200 cm^{-1} confirmed the presence of C-O str or O-H def and the absorption in the region 2800cm-1 to 3200cm-1 confirmed the presence of O-H str and aldehyde functional group. The absorption in the region 1200 to 1500cm^{-1} corresponds to C=O str. The absorptions in the region 2300 – 2900 cm-1, confirmed the presence of carbonyl (-C=O) groups. The results indicated chemical group modification for treated and untreated cotton fabrics. Hence this fabric can be utilized for various purpose and we can take it in to commertialization.

The extracellular production of silver nanoparticles by *F. oxysporum* and its antimicrobial effects were studied against S*taphylococcus aureus, Escherichia coli* and *Candida albicans.* [12]. The extremophilic actinomycete, *Thermomonospora* sp. when exposed to gold ions

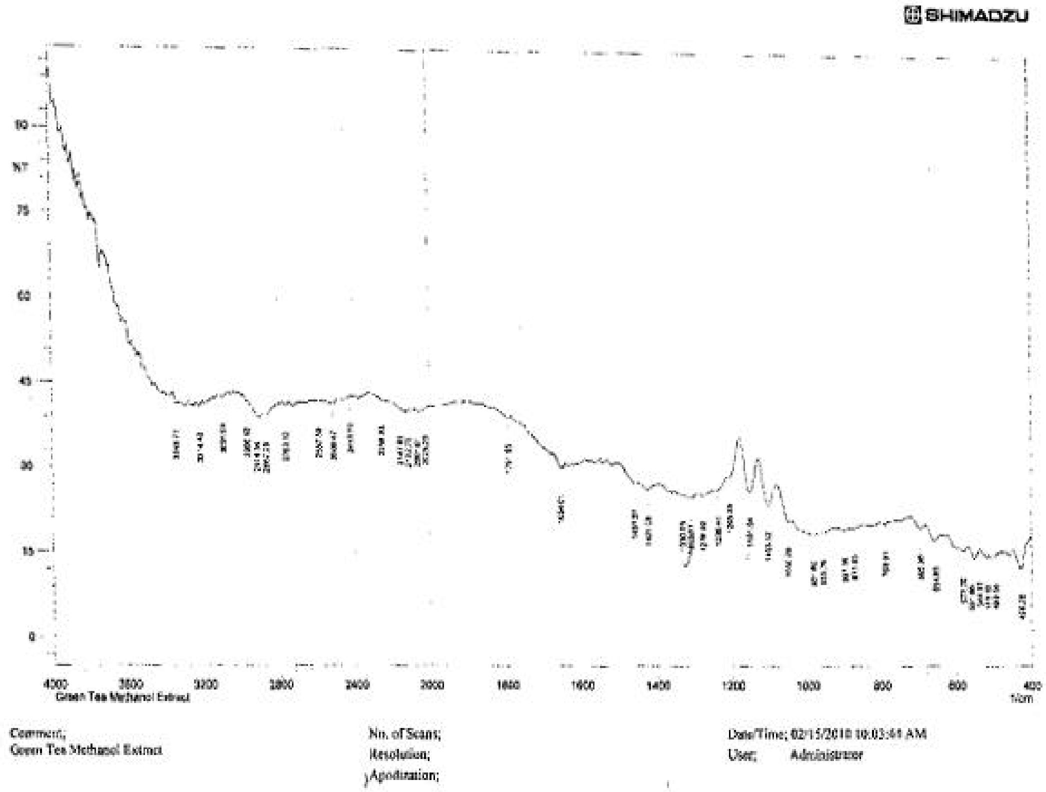

Fig. 7: FTIR analysis of copper nanofinished fabric

reduced the metal ions extracellularly, yielding gold nanoparticles with much improved polydispersity [13]. Biomass that has resulted in efficient synthesis of monodisperse gold nanoparticles. The reduction of metal ions and stabilization of the gold nanoparticles were believed to occur by an enzymatic process [14].

This is the first report on the antimicrobial effect of *Streptomyces* nanoparticle-coated fabric. The findings have shown that fabrics coated with nanoparticles are effective against *E. coli, S. aureus and A.niger*. The bacteria attached to the surface of nanoparticle-treated fabrics were killed completely. The use of a combination of antimicrobial agents may be of value in preventing the emergence of resistant bacterial strains. The presence of an antimicrobial coating on barrier clothing certainly offers additional protection to front-line healthcare workers. The present study provides scientific evidence for the antimicrobial effect of the nanoparticles against common hospital pathogens. Nanoparticle-coated fabrics were shown to have an antimicrobial effect that serves to inhibit the growth of contaminated micro-organisms. Despite the findings of this study, there are several aspects that require further investigation.

In conclusion the necessity for antimicrobial finishes in textiles is more to avoid infections, control infestation and safeguard the textile product from deterioration. The use of antimicrobials in the textile market is growing at a high speed rate. The present study demonstrated that copper sulphate may be reduced extracellularly using S*treptomyces sps* to generate stable copper nanoparticles. These particles can be incorporated in several kinds of materials such as cloths. These cloths with copper nanoparticles can be used in hospitals to prevent or to minimize infection with pathogenic bacteria. However, the elucidation of exact mechanism of nanoparticles production using living organisms needs much more experimentations.

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