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Bacterial mediated silver nanoparticles and their efficacy against MRSA

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Abstract. Bacterial mediated Silver nanoparticles is considered as an emerging Ecofriendly approach to eradicate human pathogens. This paper aims to provide the biological approach for the synthesis of silver nanoparticles from indigenously isolated bacteria. This study will be beneficial to control the nosocomial infections triggered by MRSA (Methicillin-resistant *Staphylococcus aureus*). The current study is the extracellular synthesis of silver nanoparticles by using the cell free filtrate of bacterial strains isolated from the soil. The optimization study was also carried out to obtain the maximum production of silver nanoparticles. Nanoparticles were confirmed and characterized by UV-Vis spectroscopy and Transmission Electron Microscopy (TEM) having the plasmon resonance peak between 420-450nm with 10-60nm in size range and most were spherical in shape. Synthesized silver nanoparticles showed a potential antibacterial activity against MRSA (Methicillin Resistant *Staphylococcus aureus*) *in-vitro* study. This is the green approach for the production of AgNPs, as there was no previous work done on the synthesis of silver nanoparticles by bacteria in this region of Southern Punjab, Pakistan and these nanoparticles can be used to treat nosocomial infection. These silver nanoparticles can be used in effective disease management as antimicrobial agent.

INTRODUCTION

Nanotechnology is the study, production and controlled handling of materials with a particle size < 100 nm. Among different metal nanoparticles, silver nanoparticles (AgNPs) have gained importance due to their applications in various walks of life for example in therapeutics, catalysis, bio-molecular detection and as anti-microbial, anti-platelet, anti-angiogensis agent (Arya a *et al*., 2018b). These nanoparticles can be synthesized by a number of approaches like physical, chemical and biological. Bionanoparticles synthesis is thought to be safe and eco-friendly approach as compared to other physical and chemical approaches of nanoparticles synthesis. An attractive possibility of green nanotechnology is to use microorganisms in the synthesis of silver nanoparticles (AgNPs). Microbial synthesis of nanoparticles is eco-friendly and has significant advantages over other processes since it takes place at relatively ambient temperature and pressure (Wei *et al*., 2012). Methicillin resistant *Staphylococcus aureus* (MRSA) is a global problem now a days (Ansari *et al*., 2014). New antibiotics are extremely necessary due to continuously emerging resistances. Only a few new generation antibiotics with unique mechanisms of action are available or in development in the recent years (Pasberg-Gauhl, 2014). Antibiotic producing bacteria not only from soil but also from marine environment can be a good source for the

development of new generation of antibiotics (Saleem & Iqbal, 2015). The search for new agents that are active on MRSA strains and that do not select easily for resistant strains becomes increasingly important (Tan *et al.*, 2012). So, silver nanoparticles synthesized by green chemistry can be a useful tool for the control of nosocomial infection caused by MRSA (Singh *et al.*, 2014). This study focused on the synthesis of silver nanoparticles from bacteria and the evaluation of antibacterial activity of these bacterial synthesized AgNPs. The aim of this study is to explore the hidden potential of soil bacteria of southern Punjab for the synthesis of silver nanoparticles as it is a safe approach for nanoparticles production as well as their application to control the nosocomial infection caused by MRSA all over the world in future prospects. This study is unique in this sense that there is no work has been done on the synthesis of silver nanoparticles by bacteria in green chemistry approach in this region until now.

MATERIALS AND METHODS

Isolation of silver nanoparticles synthesizing Bacteria

Soil samples (34) were collected from different regions of Southern Punjab and screened for the isolation of silver nanoparticles synthesizing bacterial strains. Total 167 bacterial strains were isolated. All bacterial strains were checked for the synthesis of silver nanoparticles, among them 5 bacterial showed positive result but only 3 bacterial strains (wus1, wus2, and wus5) were significant AgNPs synthesizer.

Bacterial identification

The physiological, morphological and biochemical properties such as Gram reaction, catalase test, indole test, sucrose fermentation test, methyl red test of strains wus1, wus2, and wus5 were performed following the method of Bergey's Manual of Determinative Bacteriology for bacterial identification.

Extracellular synthesis of silver nanoparticles

Selected strains were inoculated in LB broth in triplicates and were placed in shaking incubator for 24 hrs. After 24hrs, 5ml supernatant from each strain was taken by centrifuging it at 10,000rpm for 5min. Silver nitrate 1mM solution of equal volume was added to this supernatant and the tubes were covered with aluminium foil and placed in shaking incubator for 24hrs, at 37°C and 150rpm. Supernatant without addition of silver nitrate was taken as control maintaining the same conditions as for samples. Visual colour change from pale yellow to brown was the indication of silver nanoparticles production. The formation of silver nanoparticles is dependent on substrate concentration. As the silver nanoparticles biosynthesis is very sensitive to different physical parameters like temperature, pH and silver nitrate concentration and these parameters are helpful to determine the size, shape and yield (Arya *et al.*, 2018a). So silver nanoparticles synthesis by bacterial strains was optimized treating them with different silver nitrate concentration from 0.5 to 2mM at different pH (pH5, pH7 and pH9) and temperature (28°C, 37°C and 45°C).

Characterization and stability of silver nanoparticles

Reduction of Ag ion was confirmed by using the spectrophotometer (HALO DB-20) following the method of Arya (Arya *et al.*, 2017). The confirmation of silver nanoparticles was done by obtaining the surface plasmon resonance peak at range between 420-450nm (Arya *et al.*, 2019). Silver nanoparticles stability was checked by spectroscopy after four months.

For the characteristic study of silver nanoparticles, particles were analysed by TEM from NIBGE (Hitachi-H-7500) and their shape and size was confirmed by taking the TEM micrograph.

Analysis of antibacterial activity of silver nanoparticles against MRSA

Bacterial synthesized AgNPs further evaluated for their antibacterial potential against MRSA strains (El-Naggar *et al*., 2014). Antibacterial assay was done on 5 different strains of MRSA by using agar well diffusion method. Muller Hinton Agar (MHA) plates were prepared and 5 wells of 6mm diameter made on it by using Pasture pipette. Bacterial lawn of 24hrs old culture of MRSA strains was prepared on MHA plates by swabbing. Each well was loaded with 30µl of silver nanoparticles, then plates were incubated at 37°C, susceptibilty of MRSA strains were checked by measuring the zone of inhibition after 16hrs.

RESULTS

Out of three, two bacterial(wus1, wus2) strains were characterized as *E. coli* and one (wus5) as *Bacillus* sp. on the basis of result of thier biochemical test. In this research silver nanoparicles were biosynthesized by using the bacterial supernatant as reducing agent. Colour change from colourless pale yellow liquid to brown liquid was the indication of colloidal AgNps formation due to excitation of plasmon vibration (Figure 1). For the confirmation of AgNps synthesis spectoroscopy was performed which shown the surface plasmon resonance peak between 420-450nm. Biosynthesized silver nanopaticles stability was confirmed by spectroscopy having the surface plasmon resonance on the same peak (420-450nm) after four month.

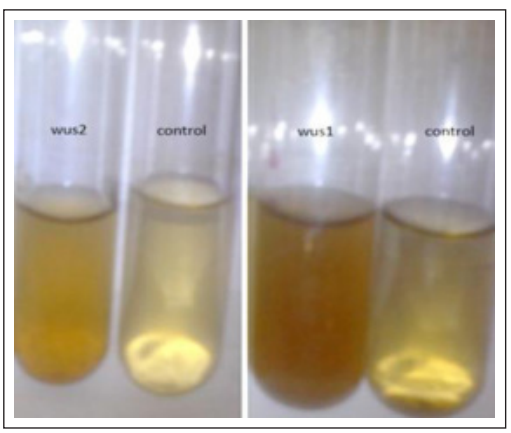

Figure 1. Biosynthesis of AgNPs by cell free supernatant of wus1 and wus2 change of colour from transparent yellow to brown is the indication of AgNPs synthesis, control is cell free supernatant without $AgNO_3$.

Optimization study of silver nanoparticles

Environmental conditions has great effect on growth and metabolism of bacterial strains. So, different physical parameters were studied to check out the optimized condition for maximum AgNPs production. Physical parameters include temperature, pH and $AgNO_3$ concentration were measured for proper growth of bacterial strains and enzyme production that is responsible for AgNPs synthesis. The optimum concentration, temperature and pH at which maximum AgNPs were synthesized was 1mM at constant temperature (37°C) and pH (7). It was confirmed by UV-visible spectroscopy by taking the spectrograph and peaks as shown in Figure 2. There was the bathochromic and hypochromic shift in the visible absorption spectrum above and below 37°C, pH 7 and 1mM $AgNO_3$ concentration which indicates the formation of larger size particles due to the agglomeration of silver clusters (Sumitha *et al*., 2018).

Characterization studies of AgNPs

TEM was done to study the morphology and measurement of AgNPs. AgNPs of different size and shape were revealed by TEM micrograph. All strains showed the different size of AgNPs ranging from 10-60nm and mostly nanoparticles were spherical in shape (Figure 3).

Antibacterial assay of AgNPs against MRSA

To evaluate the antibacterial efficacy of bacterial synthesized AgNPs, antibacterial assay of AgNPs were studied against 5 different strains of MRSA (MRSA1, MRSA2, MRSA3, MRSA4 and MRSA5). The clear zone around the each well was measured, maximum zone of inhibition (18mm) was showed by wus1 against MRSA4 and

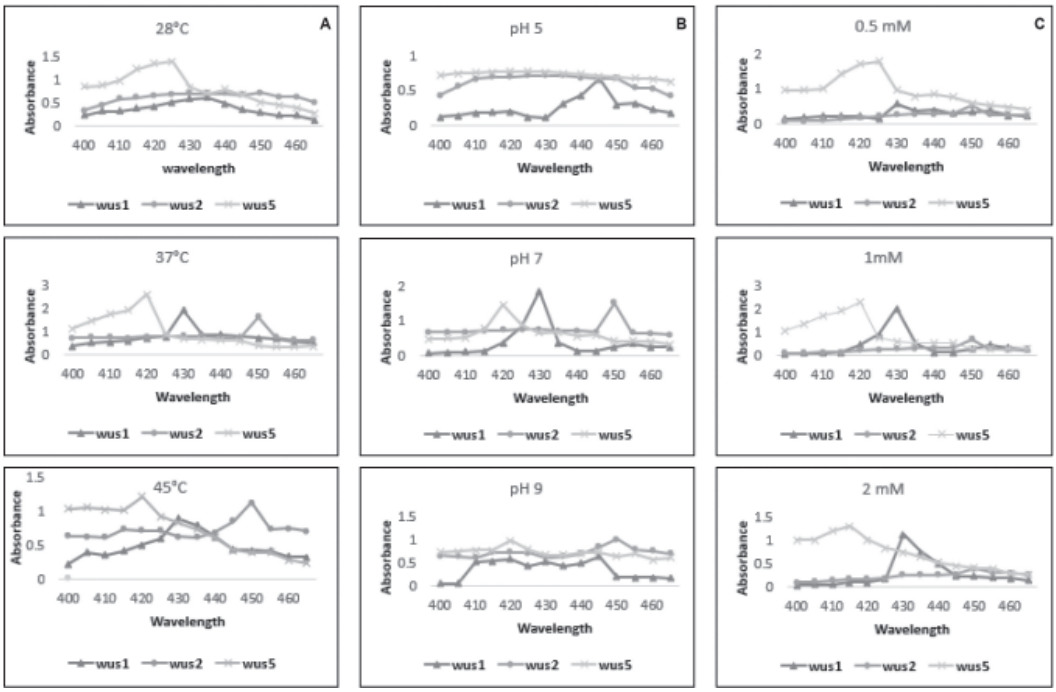

Figure 2. Optimization study of biosynthesis of AgNPs at different Temperature, pH and $AgNO_3$ concentration with other two factors constant(A)Temperature: Maximum absorbance was obtained at 37°C by wus1, wus2 and wus5(B)pH: Optimum pH for bacterial supernatant reducing agent is 7(C) $AgNO_3$ concentration: Maximum absorbance was shown at 1mM $AgNO_3$ concentration by wus1, wus2 and wus5.

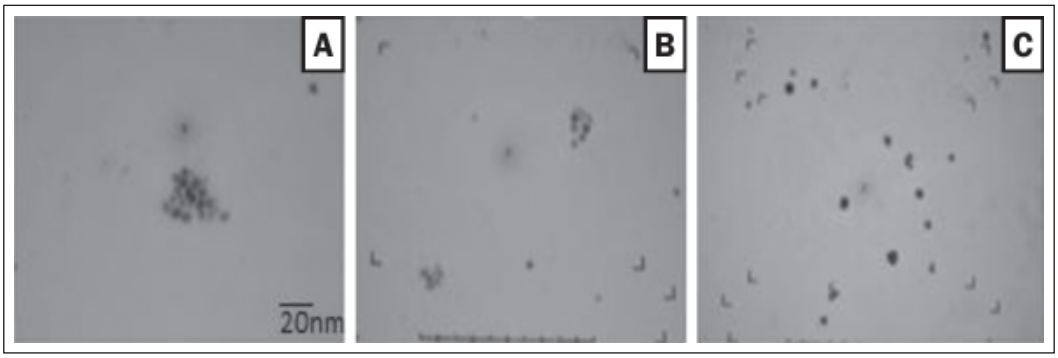

Figure 3. Transmission Electron Microscopy of biosynthesized AgNPs by wus1(A), wus2(B) and wus5(C), Scale bar: 20nm.

minimum zone of inhibition was 8mm by wus5 against MRSA2 (Table 1, Figure 4).

DISCUSSION

Although it is an attractive field of research but very few bacterial strains from a large no of bacterial sample showed positive result for the production of AgNPs as reported by Priyadarshini *et al.* (2013). In her study only one strain showed positive result for AgNPs production out of 127 strains from silver mine. The reason behind this fact is that only those bacteria which can survive on high metal concentration especially on high silver ion concentration are able to produce AgNPs (Iravani, 2014). Due to this reason a large

Table 1. Zone of inhibition of AgNPs against MRSA

AgNPs synthesizing strains	Zone of inhibition against MRSA strains for the analysis of antibacterial assay of AgNPs (mm)				
	MRSA1	MRSA2	MRSA3	MRSA4	MRSA5
wus1	15	15	14	18	13
wus2	11	12	10	10	12
wus5	9	8	12	9	12
ControlSupernatant	0	0	0	0	0
1mM $AgNO_3$ sol	0	0	0	0	0

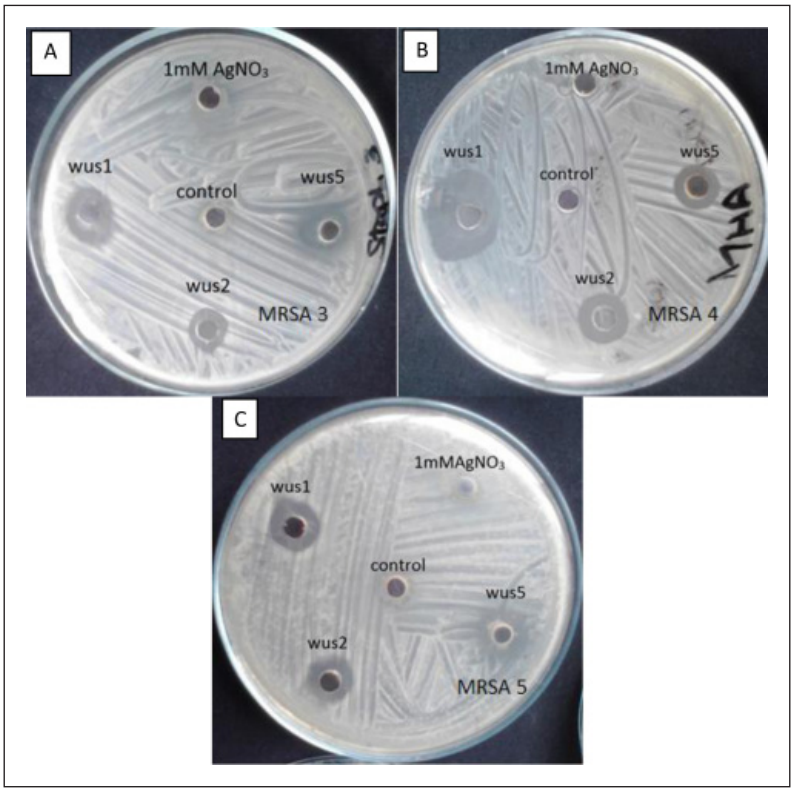

Figure 4. Antibacterial activity of AgNPs against (A) MRSA3, (B) MRSA4, (C) MRSA5.

no of soil samples (34) were collected from the different regions of southern Punjab and only 5 strains out of 167 showed the positive result for AgNPs production. Strains which showed positive result were investigated by UV-visible spectrum, which is the primary methodology for the detection of AgNPs presence.

In the optimization of physical factors 1mM concentration of silver nitrate showed the best result for AgNPs synthesis in our study, it may be due to threshold potential of isolated strains for silver ion may be 1mM or it may be explained by substrate-enzyme relation, when active sites of enzymes are occupied by higher concentration of $AgNO_3$ cannot enhance the reaction as no further sites are available for further attachment of salt (Singh *et al.*, 2013). The best pH for AgNPs production was 7, at low pH protein gets

denatured and loses its activity that's why at pH5 did not show any significant peak, while at pH9 there was mild peak. Similarly at high temperature of 45°C enzymes that is responsible for the reduction of ionic silver into silver nanoparticles cannot perform its work properly so less AgNPs are produced by these strains at 45°C.

As the MRSA is an alarming threat in health sector due to its multidrug resistance and high nosocomial infection ratio, so, these silver nanoparticles were checked for their antibacterial activity against 5 different types of MRSA (MRSA1, MRSA2, MRSA3, MRSA4 and MRSA5). Silver nanoparticles (30µL) synthesized by wus1 showed best antibacterial activity against MRSA1, MRSA2, MRSA3, MRSA4 and MRSA5 with the zone of inhibition 15mm, 15mm, 14mm, 18mm and 13mm respectively as shown in Table 1 while silver nanoparticles synthesized by the fungus showed the maximum zone of inhibition 16mm with 80µL of silver nanoparticles (Singh *et al*., 2014).

CONCLUSION

The current study is very important to explore the indigenous bacterial flora as useful bionanoparticles producer that may be further used in biomedical application and may be used as useful tool to boast the bio nanotechnology in Pakistan.

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