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EXTRACELLULAR SYNTHESIS OF SILVER NANOPARTICLES BY AN INDIGENOUS YEAST *AUREOBASIDIUM PULLULANS* RYLF 10: CHARACTERIZATION AND EVALUATION OF ANTIBACTERIAL POTENTIAL

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ABSTRACT

Abstract: *Recently, biosynthesis of silver nanoparticles is under intensive research for exploring its practical utility. The present investigation explains rapid and extracellular synthesis of silver nanoparticles using indigenous yeast Aureobasidium pullulans isolated from soil sample and their characterization employing UV-VIS spectroscopy, Transmission electron microscopy, Fourier Transform Infra-red Spectroscopy and Particle Size Analyzer. The size range of the synthesized silver nanoparticles was around 2.43 nm to 53.5 nm. The FTIR studies showed major peaks of proteins involved in the synthesis of silver nanoparticles. Further, antibacterial effect of the silver nanoparticles against multidrug resistant pathogens Escherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas sp. and Moraxella species was tested using disk diffusion method. All the resistant bacteria were found to be susceptible to the antibiotic in the presence of the silver nanoparticles.*

KEY WORDS

Silver nanoparticles, Aureobasidium pullulans, Antibacterial activity, Indigenous

I. INTRODUCTION

There is a growing need to develop clean, non-toxic and environmentally friendly procedures for synthesis and assembly of nanoparticles, which has resulted in researchers seriously looking at biological system for inspiration. There are no dearth of examples using various organisms including plants, herbal extracts, bacteria and fungi for synthesis of nanoparticles. However, production of silver nanoparticles through fungi has several advantages over other mentioned approaches. They include tolerance towards high metal nanoparticle concentration in the medium, easy management in large scale production of nanoparticles, good dispersion of nanoparticles and much higher amounts of protein expressions (Vahabi et al., 2011). The fungal mediated extracellular synthesis method has pulled in lot of interest owing to its simplicity, no further downstream processing, and lesser time utilization rather than intracellular synthesis (Mishra et al., 2011).Although silver nanoparticles are synthesized both intra and extracellularly, extracellular method of biosynthesis is more advantages due to ease of control over the environment, large-scale synthesis and straight forward processing steps (Moazeni et al., 2014; Kowshik et al., 2003).

The broad-spectrum antimicrobial properties of silver encourage its use in biomedical applications, water and air purification, food production, cosmetics, clothing,

and numerous household products. With the rapid development of nanotechnology, applications have been extended further and now silver is the engineered nanomaterial most commonly used in consumer products which exploit the antimicrobial properties of silver nanomaterials (Jones et al., 2009). Increasing incidence of microbial resistance to clinically approved classes of antibiotics and the continuing emphasis on health care costs have made a strong push towards the development of new and effective antimicrobial agents (Goffeau, 2008). The remarkable antimicrobial activity of silver nanoparticles has been well-demonstrated against a broad spectrum of Gram-positive and Gram-negative bacteria including multi drug resistant human pathogens (Ingle et al., 2008).

In the present scenario pharmaceutical and biomedical sector is facing the problem of increase in emerging pathogens with their antibiotic resistance profiles. To counter the problem of multi-drug resistant pathogens and parasites, there is a need to develop and modify the antimicrobial compounds so as to improve their microbicidal potential. Silver nanoparticles may address the problem of emerging pathogens including multidrug resistance pathogens (Raheman et al., 2011). In accordance to the above, present study aimed at exploring the silver nanoparticles producing potential of an indigenous yeast isolated from soil (Yadav et al., 2012) *Aureobasidium pullulans* and to screen out the antibacterial properties of nanoparticles being biosynthesized and to study the effect of fungal nanoparticles antibacterial property. Besides, the study also reports the antibacterial efficacy of few commercial antibacterial antibiotics.

II. MATERIAL AND METHODS

Collection of an indigenous fungal organism

The culture of fungal test organism *Aureobasidium pullulans* was obtained from the Department of Microbiology, Panjab University, Chandigarh and maintained on Potato Dextrose Agar (PDA) slants.

Cultivation of fungal biomass

The *Aureobasidium pullulans* culture was inoculated into 100 mL potato dextrose broth (PDB) in a 250 ml Erlenmeyer flask. Flask was incubated at 28 °C ±0.5 °C under shaking at 150 rpm for 7 days. After incubation, the fungal biomass was harvested from fermentation broth by centrifuging the broth at 5000 rpm for 20 minutes. The biomass so obtained was washed 3-4 times with distilled water so as to remove medium particles attached to it.

Synthesis of silver nanoparticles (SnPs) by fungal biomass

The formation of silver nanoparticles by *Aureobasidium pullulans* biomass was done as per methods of Hemanth et al (2010). In this method the 10 gm of biomass was suspended in 100 ml of deionised water in 250 ml Erlenmeyer flasks. Flask was kept at 27°C, under shaking conditions at 150 rpm for 72 hours. After respective incubation, the biomass was separated by centrifuging the broth at 5000 rpm for 20 minutes to obtain the cell free filtrate. 0.5 mL of 1/10 M $AgNO_3$ was added to 49.5 ml of cell free filtrate so as to get the 1mM volume as the final concentration of $AgNO_3$. The cell free filterate (without $AgNO_3$) was maintained as control. Progress of the reaction that is the formation of the silver nanoparticles was monitored by visual inspection and UV-VIS spectroscopy under the wavelength scan of 200-800 nm.

Evaluation of antibacterial activity of extracellular silver nanoparticles

The evaluation of antibacterial activity of the above synthesized silver nanoparticles was done by disk diffusion method of Kirby Bauer (1961). In this method, first of all the different bacterial test pathogens (*Escherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas* and *Moraxella* species) were inoculated separately by spreading on to nutrient agar plates then the sterilized Whatman filter paper disks (of 5mm size) impregnated with concentrated 10 µL of silver nanoparticle solution was placed on to the plates under aseptic conditions and incubated at 37±1°C for 24 hrs and observed for the formation of growth of inhibition (if any). The disks impregnated only with 30 µL of $AgNO_3$ and antibiotic discs of Kanamycin and Chloramphenicol were used as control.

Evaluation of antibacterial efficiency of commercial antibiotics in combination with extracellular SnPs

The effect of antibacterial activity of different commercial antibiotics (Chloramphenicol and Kanamycin) in combination with silver nanoparticles produced by *Aureobasidium pullulans* on different bacterial test pathogens was also analyzed separately and compared with control (impregnated each separately with test antibiotics and the $AgNO_3$ alone). The antibacterial effect was investigated against both the Gram positive (*Staphylococcus aureus*) and Gram-

negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas* and *Moraxella* sp.) by as per disk diffusion method of Kirby - Bauer, 1961. The silver nanoparticle + antibiotic discs were prepared by impregnating the sterlizedWhatman filter paper discs (of 5mm size) with concentrated 30µL solution of each silver nanoparticles and the test antibiotics separately. Similarly, the control disks were prepared by impregnating the discs each with 30µL of test antibiotics and $AgNO_3$ alone. The test antibiotics used were chloramphenicol and kanamycin

The experiments were performed separately in different nutrient agar plates each inoculated with single bacterial test pathogen and then inoculated with different disks containing silver nanoparticles produced by *A.pullulans* + antibiotics and control disks containing antibiotic and $AgNO_3$ alone. The plates were incubated at 37±1 ^oC for 24 hrs and observed for the formation of zone of inhibition (if any). The diameter of the inhibition zone (mm) around different silver nanoparticle + antibiotic disks and the control disks with antibiotics and $AgNO_3$ alone against test pathogens were calculated by measuring increase in fold area.

The increase in fold area was assessed as per Birla et al., 2009 calculating the mean surface area of the inhibition zone of each antibiotic (A) and antibiotic+silver nanoparticle (B). The fold increase area of bacteria was calculated by the equation $(B^2-A^2)/A^2$ where 'A' and 'B' were zones of inhibition for antibiotic and antibiotic + silver nanoparticles, respectively.

Characterization of fungal silver nanoparticles

The characterization of size and shape of fungal silver nanoparticles was done by analyzing the nanoparticles by transmission electron microscopy (TEM).

FTIR spectroscopy is widely used to study the nature of surface adsorbents in nanoparticles. The filtrate containing silver nanoparticles was centrifuged and the pellet was used for the FTIR. FTIR spectra of nanoparticles were recorded with a PerkinElmer spectrum 400 spectrometer over KBr Pellet (Mukherjee *et al*, 2001).

Particle Size Analysis (PSA) study was done with particle size analyzer to determine the percentile of size differences of the fungal silver nanoparticles.

III. RESULTS AND DISCUSSION

Synthesis of fungal silver nanoparticles

The synthesis of silver nanoparticles by the *Aureobasidium pullulans*, test culture (Figure no 1) was detected by the change in the colour from transparent/yellow to dark brown (Figure no 2) of the suspension containing cell free filterate and $AgNO_3$ as a result of the fungal mediated reaction with $AgNO_3$ for the formation of silver nanoparticles which is due to reduction of silver ions to silver nanoparticles (i.e. Ag^+ to Ag^0) within 24 hours and which goes on increasing with increase in incubation up to 120 hrs.

Figure No 1: Growth of *Aureobasidium pullulans* (test culture) on PDA plate showing characteristic black coloured colonies.

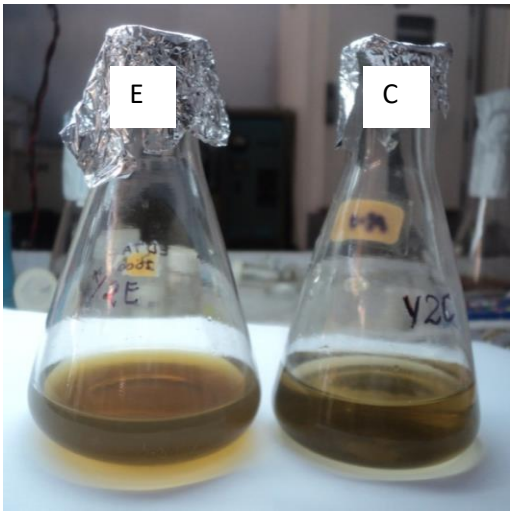


Figure No 2: Flasks showing the formation of silver nanoparticles by *Aureobasidium pullulans* as observed by the change in colour of reaction mixture in experimental (E) flasks (dark brown) and no change in colour was observed in control flask containing only the cell free supernatant. (C).

The synthesis of fungal SnP was also confirmed by UV-VIS spectroscopy analysis of the suspension containing the synthesized nanoparticles in which the peak obtained at the end of 5th day (1.194) by all the silver nanoparticles was comparatively good and was in range of 380-480 nm which is the defined range of the silver nanoparticles (Figure no 3; Table 1). The colour change from yellowish to dark-brown indicates the formation of silver nanoparticles in the reaction mixture. The appearance of brown colour was due to the excitation of surface plasmon vibrations (Ahmad *et al* 2003; Vahabi *et al*., 2011; Duran *et al*., 2007). Different research groups have also used UV-Visible spectrophotometry to track the progress of nanoparticle production from $AgNO_3$. Production of silver nanoparticles is indicated by a peak in 400-500 nm region due to surface plasmon resonance.

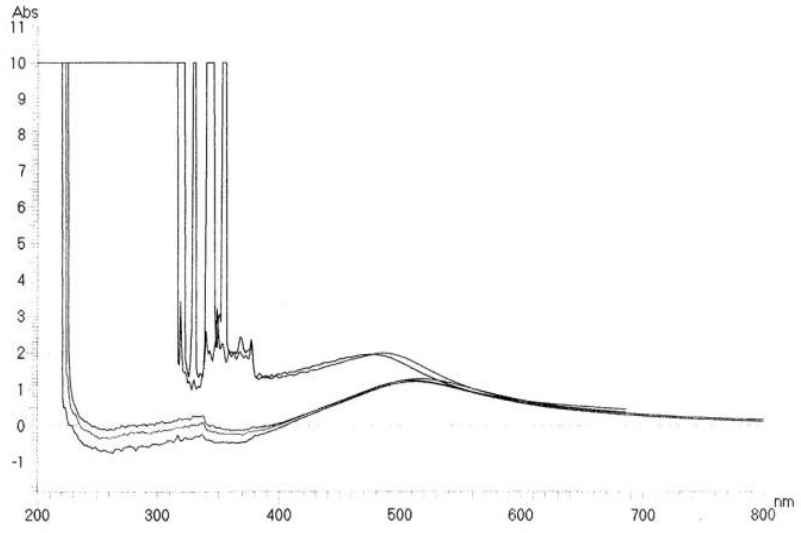


Figure No 3 Peaks of silver nanoparticles synthesized by *A. pullulans* obtained by UV –VIS spectroscopical analysis after 24, 72, 96 and 120 hours; Absorption peak increasing with time.

Table No 1: Day wise UV-VIS spectroscopy details of silver nanoparticles produced by *A.pullulans*

Time (in hours)	Apex(nm)	Height(absorption)
24	475.0	1.939
72	484.0	1.970
96	510.0	1.228
120	513.0	1.194
240	517.0	1.269

Characterization of fungal silver nanoparticles

TEM study of SnP

The silver nanoparticles biosynthesized by the indigenous isolate *Aureobasidium pullulans* were deposited on carbon coated copper grids and then analyzed by TEM (Transmission Electron Microscopy). The size of the nanoparticles is highly variable ranging from 2.43 nm to 53.5 nm (Figure no 4). The shape was also variable but mostly were spherical and poly disperse. The images show individual silver nanoparticles as well as aggregates.

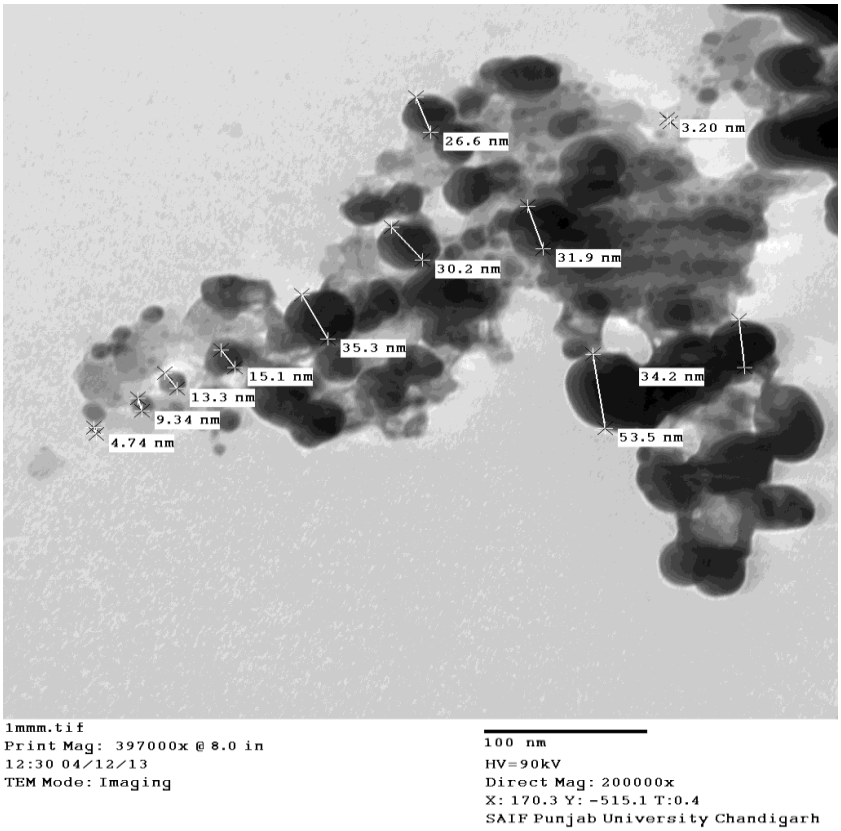


Figure No 4: TEM image showing individual and aggregates of SnP (synthesized by *A. Pullulans*) polydisperse, with highly variable size

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR is a powerful tool for identifying types of chemical bonds in a molecule by producing an Infrared absorption spectrum that is like a molecular "fingerprint". The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. The main goal of FTIR in this study was to determine the presence of functional groups in the sample. The FTIR measurement can also be utilized to study the presence of a protein molecule in the solution, as the FTIR spectra in the 1400–1700 cm^{-1} region provides information about the presence of "*C=O*" and "*N-H*" groups (Senapati *et al.,* 2005).

The amide linkages between amino acid residues in polypeptides and proteins give rise to well-known signatures in the infrared region of the electromagnetic spectrum. The positions of the amide I and II bands in the FTIR spectra of proteins are a sensitive indicator of conformational changes in the protein-secondary structure (Mukherjee *et al.,* 2001, Okansen *et al.,* 2000). The FTIR spectrum was recorded from a drop-coated film of the silver nanoparticle-fungus reaction mixture on *Si* (111) substrate. The FTIR spectra show peaks at 1638 cm^{-1} and 1458 cm^{-1}. These are due to *–C=O* and *N-H* stretch vibrations present in the amide linkages of the proteins, respectively (Figure no 4). Similar peaks have been reported by Vahabi *et al.,* 2011 in FTIR spectra of silver nanoparticles biosynthesized by fungus *Trichoderma reesei*. Other peaks in the FTIR spectra of Silver Nanoparticles biosynthesized by *A.pullulans* indicates presence of *–NH* stretch vibrations of primary amines (3400 cm^{-1}), *-CH* asymmetric and symmetric stretch (2927cm^{-1}). The positions of the bands

corresponding to *–C=O* and *N-H* stretch vibrations present in the amide linkages of the proteins are close to that reported in literature for native proteins (Mukherjee *et al.,* 2001, Okansen *et al.,* 2000). Thus, the FTIR measurement indicates that the secondary structure of proteins is not affected because of its interaction with Ag^{+} ions or nanoparticles.

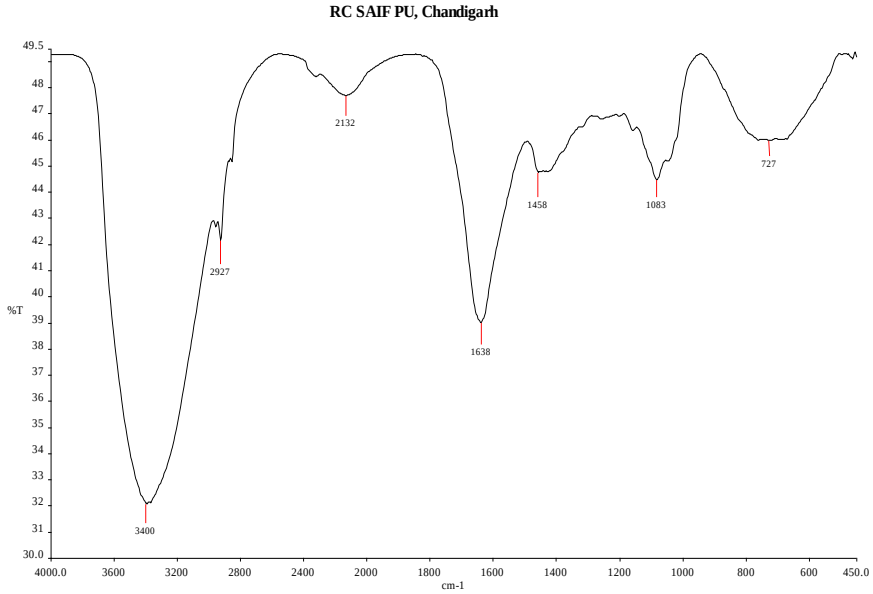


Figure No 4: FTIR of silver nanoparticles synthesized by *A. pullulans*

Particle Size Analysis

Particle Size Analysis was performed using Malvern Hydro 2000S instrument. The principal of PSA (Particle Size Analysis) is that when a focused laser beam is passed through the particle preparation, the particles will scatter light at angles inversely proportional to their size. The map of scattering intensity versus angle is the primary source of information used to calculate the particle size. In case of nanoparticles biosynthesized by *Aureobasidium pullulans,* 10 % particles of the total population was less than 72 nm (i.e. d (0.1) =72 nm). The median size of the particle population was 115 nm (which means that 50% of the particles are smaller than this diameter. Thus, particle size analysis indicates great variation in the size of silver nanoparticles as was pointed by TEM. Also, there are reports of mycosynthesis of silver nanoparticles of size ranging from 1-100 nm (Vahabi *et al*.,2011, Duran *et al*.,2007, Sawle *et al*.,2008).

It was concluded that the test fungal isolate have the potential to biosynthesize silver nanoparticles of a varying size range.

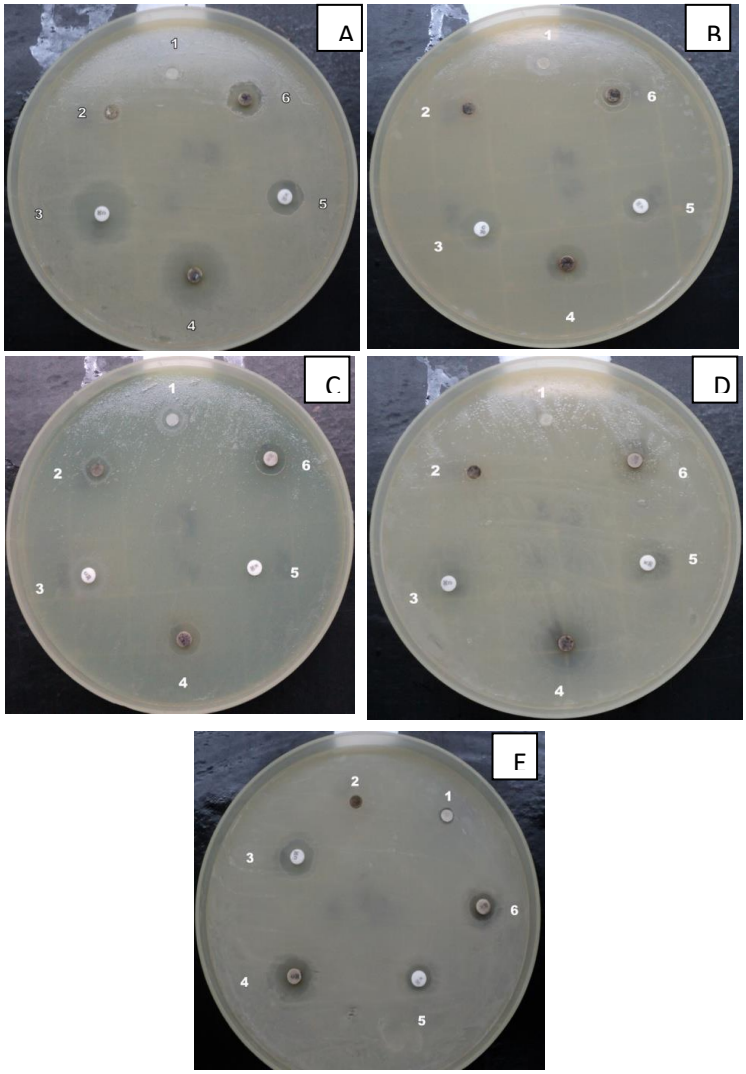
Antibacterial activity of fungal silver nanoparticles

The antibacterial activity of the silver nanoparticles produced by the test fungi was determined as per standard disc diffusion method of Kirby-Bauer (1961). The antibacterial activity was measured as the size (diameter) of the zone of inhibition in mm. The bigger the zone of inhibition, the higher the antibacterial activity. The results revealed the presence of antibacterial activity in the silver nanoparticles produced by *A. pullulans* with the highest zone of inhibition in mm reported against *E.coli and Moraxella* sp. (10 mm) followed by species of *Pseudomonas* (9 mm) and *S.typhi* (8mm). However, the least activity was found against *S.aureus* (6.6mm) (Table 2). This can be concluded as that the biosynthesized silver nanoparticles were comparatively more effective against Gram negative bacteria as compared to Gram positive bacteria (*Staphylococcus aureus)*. This difference in the activity of silver nanoparticles against Gram positive and negative bacteria may be attributed to structural differences in cell wall composition. The Gram-negative bacteria have a layer of lipopolysaccharides at the exterior, followed underneath by a thin (7–8 nm) layer of peptidoglycan (Fayaz *et al.,* 2009; Madigan *et al.,* 2005). Although the lipopolysaccharides are composed of covalently linked lipids and polysaccharides, there is a lack of strength and rigidity. The negative charges on lipopolysaccharides may be attracted towards the weak positive charge available on AgNPs (Sui *et al*., 2006). On the other hand, the cell wall in Gram-positive bacteria is composed of a thick layer (20–80 nm) of peptidoglycan (polymer of N-

acetyl muramic acid and N- acetyl glucosamine) consisting of linear polysaccharide chains cross-linked by short peptides to form a three-dimensional rigid structure (Baron *et al.,* 1996; Fayaz *et al.,* (2009); Sondi *et al.,* (2004); Ruparelia *et al.,* (2008). The rigidity and extended cross-linking not only endow the cell walls with fewer anchoring sites for the AgNPs but also make them difficult to penetrate. Thus, the silver nanoparticles in this investigation biosynthesized by *A. Pullulans* have the potential to be used as antibacterials especially against the Gram negative bacteria.

Table No 2: Zone of inhibition in diameter (mm) obtained by silver nanoparticles Synthesized by *A. Pullulans* against five different test bacteria.

Test Bacteria	*A. pullulans*
E.coli	10
Pseudomonas sp.	9
S.aureus	6.6
S.typhi	8
*Moraxella*sp.	10



1: $AgNO_3$; 2: Silver Nanoparticles ; 3 : Chloramphenicol disc (30mcg/disc); 4 : Chloramphenicol disc with Nanoparticles ; 5: Kanamycin disc (30mcg/disc) ; 6 :Kanamycin disc with nanoparticles

Figure No 5: Antibacterial activity of nanoparticles and combination of nanoparticles and Antibiotics against A: *E.coli* , B: *Moraxella* , C : *Pseudomonas* , D: *S.aureus* , E :*S.typhi*

The result pertaining to the effect of fungal silver nanoparticles on different commercial antibiotics (Chloramphenicol and Kanamycin) in combination revealed an overall increase in the efficiency of antibacterial activity of both the antibiotics.

Effect on antibacterial activity of Chloramphenicol in combination with fungal silver nanoparticles

The result obtained on the efficacy of antibacterial activity of chloramphenicol when it was used in combination with silver nanoparticles produced by *A.pullulans* against 5 different bacterial pathogens (*E.coli , Pseudomonas sp. , Moraxella sp., S.aureus* and *S.typhi*) reveals an overall increase in fold areas (zone of inhibition) which indicates the increase in antibacterial potential of chloramphenicol. However, the maximum increase in fold area of inhibition (6.71) was obtained against *E.Coli* when chloramphenicol was combined with the silver nanoparticle produced by *Aureobasidium pullulans*. The nanoparticles produced were effective against all bacterial pathogens but the E. coli was found to be the more sensitive against them.

Table No 3: Increase in antibacterial efficiency of Chloramphenicol in combination with silver nanoparticles

Test Pathogen	Zone of inhibition (nanoparticle) (mm)	Zone of inhibition of antibiotic (mm)	Zone of inhibition of antibiotic with nanoparticle(mm)	{Zone of inhibition of antibiotic with nanoparticle(mm)}^{2}- {Zone of inhibition of antibiotic (mm)}^{2}	Increase in fold area
E.coli	13	9	25	544	6.716049
Pseudomonas sp.	10	15	19	136	0.604444
S.aureus	6	12	19	217	1.506944
S.typhii	7	20	25	225	0.5625
Moraxella sp.	14	15	17	64	0.284444

It was observed that out of 5 bacterial pathogens used under study, *E. coli* was found to be the least susceptible with chloramphenicol (the antibiotic) and the silver nanoparticle alone respectively. But when both, the antibiotic and the silver nanoparticles were used in combination, its (*E. coli*) susceptibility increased the most as evidenced by the Table no 2; Figure no 6.

Effect on antibacterial activity of Kanamycin in combination with fungal silver nanoparticle

The result obtained on the efficacy of antibacterial activity of kanamycin when it was used in combination with silver nanoparticles produced by *A.pullulans* against 5 different bacterial pathogens (*E.coli , S.aureus , S.typhi , Moraxella* sp. and *Pseudomonas* sp.) reveals an overall increase in fold areas (zone of inhibition) which indicates the increase in antibacterial potential of kanamycin. However, the maximum increase in fold area of inhibition (4.29) was obtained against *E.Coli* when Kanamycin was combined with the silver nanoparticles. The trend of result obtained reveals that the *E. coli* was the most susceptible against the nanoparticles produced by the test fungus in combination with kanamycin.

Table No 4: Increase in antibacterial efficiency of Kanamycin in combination with silver nanoparticles

Test Pathogen	Zone of inhibition (nanoparticle) (mm)	Zone of inhibition of antibiotic (mm) (A)	Zone of inhibition of antibiotic+nanoparticle(mm) (B)	{Zone of inhibition of antibiotic with nanoparticle(mm)}^{2}- {Zone of inhibition of antibiotic (mm)}^{2}	Increase in fold area
E.coli	10	10	23	429	4.29
Pseudomonas sp.	9	9	12	63	0.77
S.aureus	8	8	14	132	2.06
S.typhii	10	10	13	69	0.69
Moraxella	10	10	20	300	3

As observed with the screening of antibacterial activity of silver nanoparticles in combination with kanamycin, enhancement of zone of inhibition was more prominent against gram-negative bacteria than gram-positive.

IV. CONCLUSION

Thus, it was concluded that comparatively, the antibacterial efficacy of Chloramphenicol and Kanamycin (antibiotics) separately in combination with silver nanoparticles, indicated that in case of *E.Coli* , higher inhibition zones were obtained in presence of chloramphenicol and silver nanoparticles as compared to combination of Silver nanoparticles with kanamycin . In case of other bacteria - *S.aureus*,*S.typhi* ,*Moraxella* and *Pseudomonas* inhibition zone was enhanced more by the combination of nanoparticles with Kanamycin as compared to Chloramphenicol.

REFERENCES

[1]. Ahmad A, Mukherjee P, Mandal D, Senapati S, Khan MI, Kumar R, Sastry M (2003) Extracellular biosynthesis of silver nanoparticles using the fungus *Fusariumoxysporum*. Colloids Surf B Biointerfaces 28:313–318.

[2]. Baron S (1996) Medical microbiology. 4th ed. Galveston: University of Texas Medical Branch.

[3]. Birla SS, Tiwari VV, Gade AK, Ingle AP, Yadav AP, Rai MK (2009) Fabrication of silver nanoparticles by Phomaglomerata and its combined effect against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus. Lett in App Microbio 48(2): 173-179.

[4]. Duran N, Marcato PD, Alves OL, DeSouza G, Esposito E (2005) Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusariumoxysporum*strains. *Journal of* Nanobiotechnology 3: 1-8.

[5]. Fayaz AM,Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R (2009) Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram negative bacteria. Nanomedicine 6(1): 103–109.

[6]. Gajbhiye M, Kesharwani J, Ingle A, Gade A, Rai M (2009) Fungus mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomedicine Nanotechnology, Biology and Medicine 5: 382-386.

[7]. Goffeau A. (2008) Drug resistance: the fight against fungi. Nature 452: 541-542.

[8]. Hemanth Naveen KS, Gaurav K, Karthik L, BhaskaraRao KV (2010) Extracellular biosynthesis of silver nanoparticles using the filamentous fungus *Penicillium* sp. Archives of Applied Science Research 2(6):161-167.

[9]. Ingle A.. Gade A., Pierrat S., Sonnichsen C. , .Rai M (2008) Mycosynthesis of silver nanoparticles using the fungus Fusarium acuminatum and its activity against some human pathogenic bacteria. Curr. Nanosci. 4: 141-144.

[10]. Jones CM, Hoek EMV (2010) A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J Nanopart Res (2010) 12:1531–1551.

[11]. Kowshik M, Ashtaputre S, Kharrazi S, Vogel W, Urban J, Kulkarni SK (2003) Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. Nanotechnology, 14(1):95.

[12]. Li P, LiJ, Wu C, WuQ, Jian Li J (2005) Synergistic antibacterial effects of β- lactam antibiotic combined with silver nanoparticles. Nanotechnology 16: 1912–1917.

[13]. Madigan M, Martinko J (2005) Brock biology of microorganisms. 11th ed.Englewood Cliffs, Prentice Hall.

[14]. Mishra A, Tripathy S., Wahab R., Jeong S.-H, Hwang I, Yang Y.-B (2011). Microbial synthesis of gold nanoparticles using the fungus *Penicillium brevicompactum* and their cytotoxic effects against mouse mayo blast cancer C2C12 cells. Appl. Microbiol. Biotechnol. 92: 617-630.

[15]. Moazeni M, Shahverdi AR, Nabili M, Noorbakhsh F, Rezaie S. (2014) Green synthesis of silver nanoparticles: The reasons for and against Aspergillus parasiticus. Nanomed J. 1(4):267–75.

[16]. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Ramani R, Parischa R, Ajayakumar PV, Alam M, Sastry M, Kumar R (2001) Bioreduction of $AuCl_4^-$ ions by the fungus *Verticillium* sp and surface trapping of the gold nanoparticles formed. Angewante Chemie International Edition 40: 3585-3588.

[17]. Oksanen T, Pere J, Paavilainen L, Buchert J, Viikari L (2000) Treatment of recycled kraft pulps with *Trichodermareesei*hemicellulases and cellulases. J Biotechnol 78: 39–48.

[18]. Raheman F, Deshmukh S, Ingle A, Gade A, Rai M (2011) Novel antimicrobial agent synthesized from an endophytic fungus *Pestalotia*sp. isolated from leaves of *Syzygiumcumini*(L). Nano Biomed Eng 3(3): 174-178.

[19]. Rai M, Yadav A, Gade A (2008) Current trends in Phytosynthesis of metal nanoparticles. Critical Reviews in Biotechnology 28(4): 277-284.

[20]. Rai MK, Gajbhiye M, Kesharwani J, Ingle A, Gade A (2009) Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomedicine: Nanotechnology, Biology, and Medicine 5: 382–386.

[21]. Rai MK, Yadav A, Gade A (2009) Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances 27(1):76–82

[22]. Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S (2008) Strain specificity in antimicrobial activity of silver and copper nanoparticles. ActaBiomateriala 4(3): 707-716.

[23]. Sawle BD, Salimath B, Deshpande R, Bedre MD, Prabhakar BKand Venkataraman A (2008) Biosynthesis and stabilization of Au and Au–Ag alloy nanoparticles by fungus, *Fusariumsemitectum.* SciTechnolAdv Mater 9:1-6.

[24]. Senapati S (2005) Biosynthesis and immobilization of nanoparticles and their applications. Ph.D. thesis, University of Pune.

[25]. Senapati S, Ahmad A, Khan MI, Sastry M, Kumar R (2005) Extracellular biosynthesis of bimetallic Au-Ag alloy nanoparticles. Small 1(5): 517-20.

[26]. Shankar SS, Ahmad A, Pasricha R, Sastry M (2003) Bioreduction of chloroaurate ions by *Geranium* leaves and its endophytic fungus yields gold nanoparticles of different shapes. Journal of Material Chemistry 13: 1822-1826.

[27]. Shankar SS, Ahmad A, Rai A, Sastry M (2004) Rapid synthesis of Au Ag and bimetallic Au core-Ag shell nanoparticles by using neem (*Azadirachta indica*) leaf broth. Journal of Colloids and Interface Science 275(5): 496-502.

[28]. Sondi I, Sondi BS (2004) Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. Journal of Colloid and Interface Science 275: 177–182.

[29]. Southam G, Beveridge TJ (1994) The *in vitro* formation of placer gold by bacteria. GeochCosmochActa 58: 4527-4530.

[30]. Sriram MI, Manikanth SB, Kalishwarlal K, Gurunathan S (2010) Antitumour activity of silver nanoparticles in Dalton's lymphoma ascites tumor model. Int J Nanomedicine 5: 753–762.

[31]. Sui ZM, Chen X, Wang LY, Xu LM, Zhuang WC, Chai YC (2006) Capping effect of CTAB on positively charged Ag nanoparticles. Physica E 33:308-14.

[32]. Vahabi K, Mansoori GA, Karimi S (2011) Biosynthesis of Silver Nanoparticles by Fungus TrichodermaReesei (A Route for Large-Scale Production of AgNPs).Insciences J 1(1) : 65-79.

[33]. Yadava KL, Rahi DK, Soni SK, and Rahib S, Diversity of exopolysaccharide producing fungi from foot hills of shivalik ranges of chandigarh capital region. Res Biotechol; 3(4):11–18, 2012.

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