ISSN 2348 – 8034
Impact Factor- 4.022

GLOBAL JOURNAL OF ENGINEERING SCIENCE AND RESEARCHES

AN OVERVIEW: MECHANISTIC APPROACH OF SILVER NANOPARTICLE MYCOFABRICATION AND ITS PROSPECTIVE APPLICATIONS

Nida Tabassum Khan,[*1] Maham Jamil Khan[2] & Jibran Jameel[3]
[*1,2&3] Department of Biotechnology, Faculty of Life Sciences and Informatics, Balochistan University of Information Technology Engineering and Management Sciences,(BUITEMS),Quetta, Pakistan

ABSTRACT
Silver nanoparticles is gaining momentum because of its unique physiochemical properties. Therefore biological methods have been employed for its synthesis. Among such methods mycofabrication is becoming popular which make use of eukaryotic fungi to produce nanoparticles of different morphology either extracellularly or intracellular with the help of an enzyme nitrate reductase

***Keywords**: Silver nanoparticles; Extracellular; Intracellular; Mycofabrication.*

I. INTRODUCTION

Cell wall of the eukaryotic multicellular fungi is composed of complex sugar molecules such as carbohydrates i.e. polysaccharides and glycoproteins that contributes in metal ion reduction to form nanoparticles of silver. During initial phase of bioreduction, silver metal ions get localized on fungal cell surface because of electrostatic force of attraction exhibited by positively charged substitute groups in proteins that resides within the fungal cell wall. Second phase of bioreduction involves the formation of silver metal ion aggregates with the help of reduction-specific enzymes resulting in the fabrication of silver nanoparticles [1].Transmission electron microscopy of the fungus confirms amalgamation of silver nanoparticles within the cytoplasm and as well on the plasma membrane. This shows the possibility that silver metal ions are capable of diffusing through the cell wall of the fungal cell followed by enzymatic reduction .Smaller nanoparticles get trapped within the cytoplasm by diffusing through the fungal cell wall [2].

II. MECHANISTIC ASPECT OF BIOGENIC SILVER NANOPARTICLES SYNTHESIS

The mechanistic aspect behind the assembly of silver nanoparticles was not clear before [3]but later it was stated that enzyme nitrate reductase , an NADH-dependent protein involved in the reduction of silver ions .As confirmed in *Fusarium* speciesthat through the action of enzyme reductase and electron shuttle quinones silver nanoparticles were produced [4,5].Depending on the location where silver nano crystals are formed either outside or inside the fungal cell, silver nanoparticle synthesis can be divided into two types:

1. Extracellular mycosynthesis
2. Intracellular mycosynthesis

Extracellular mycosynthesis of Silver nanoparticles

Mechanistic aspect of extracellular mycosynthesis
Interaction between silver metal ions and released proteins mainly reductase results in the succeeding accumulation of extracellular silver nanoparticles in the external environment [6].As nitrate reductase assay results revealed that enzyme reductase or electron shuttle quinones or both contributes in the extracellular amalgamation of silver nanoparticles[5] .For example fungus *F. semitectum* was reported to be the extracellular producer of stable silver nanoparticle when 1mM of silver substrate was exposed to the fungal filtrate [7]. Another example of extracellular silver nanoparticle have been reported in *Aspergillus sp*[8].

Intracellular mycosynthesis of Silver Nanoparticles

Mechanistic aspect of intracellular mycosynthesis

Electrostatic binding of heavy metals to the fungal cell wall with the help of proteins or enzymes secreted by the fungus on its surface is responsible for the intracellular mycosynthesis of silver nanoparticle. Furthermore, reduction of metal ions were carried by these enzymes or proteins molecules localized in fungal outermost wall. This results in aggregation of the metal ions forming a complex which then in return produces the nanoparticles. For exampleenzyme involved in intracellular mycosynthesis of silver nanoparticle is NADPH dependent known as nitrate reductase i.e. having capability to reduce metal ions [9]. Bioreduction of silver ions by this particular enzymes lead to the production of silver nanoparticles. Production of silver nano crystals requires hydroxyquinoline which acts as an electron shuttle for silver ions reduction and also the reduction of a-NADPH to a-NADP+. Another study reported fabrication of capped Silver nanoparticles using fungus proteins of *Coriolus versicolor* [10].

III. NITRATE REDUCTASE

There are numerous evidences to depict the importance of nitrate reductase in the assembly of silver nanoparticle. The role of intracellularnitrate reductase for the synthesis of Silver nanoparticlesof size 50 nm from *Halococcus salifodiane*have been well documented [11].Another evidence was *Trichoderma virens* mediated silver nanoparticles amalgamation which involves nitrate reductase mediatedsilver ion reduction[12].Similarily,nitrate reductase mediated silver ion reduction in the culture supernatant of *Nocardiopsis sp* was also reported[13].Presence of nitrate reductase was depicted in the cell free extract of Neuraspora intermedia during sliver nanocrystals formationand showed significant antibacterial activity [14].In recent times, enzyme nitrate reductase was isolated from the fungus *Fusarium oxysporum* on specific medium by ion exchange chromatography and ultrafiltration technique for the production of silver nanoparticle. In addition using gelatin as a capping agent nitrate reductase mediated fabrication of silver nanoparticle was dependent on NADPH [15].In the light of the above mentioned examples, nitrate reductase assay was developed .When the discs were exposed to fungal filtrate, color change from white to reddish suggesting the presence of nitrate reductase [16]. So, it can be concluded thatnitrate reductase is NADH dependent enzyme crucial for silver ion reduction in case of fungi.

IV. APPLICATIONS OF BIOGENIC SILVER NANOPARTICLES

Silver nanoparticles is the most popular metallic nanoparticle[17,18,19,20] because of their unique electromagnetic, optical, catalytic, anti-proliferative and antimicrobial effectscompared with other metal nanoparticles[21,22,23,24]. Some of the well-known applications of mycofabricated silver nanoparticle s are given below:

1. **Anti-bacterial effects**

Silver in small concentrations is tolerable by human cells but such concentration is deadly for pathogens.Thus exploiting this property silver nanoparticles can be employed as antimicrobial agents [25].For example pathogenic Gram positive bacteria, *Staphylococcus aureus* has been used to synthesize Silver nanoparticles that showed anti-bacterial activity against methicillin-resistant *Staphylococcus epidermidis ,methicillin-resistant S. aureus*, and *Streptococcus pyogenes* where as a narrow spectrum of resistance was seen gainst *Klebsiella pneumoniae* and *Salmonella.*[26] Another example demonstrating antibacterial activity against*Pseudomonas aeruginosa* isolated from burnt patients [27]. Besides, several studies including antibacterial effect of mycosynthesized Silver nanoparticles against pathogenic bacteria like*E. coli, Agrobacterium tumifaciens, Magnaporthe oryzae* [28],*Psuedomonas fluorescens*[29],*S. aureus, S. typhi* [16],*P. aeruginosa, S. aureus* [29] has been documented. Antimicrobialspectrum of silver nanoparticles is greatly influenced by its pattern of distribution, their size range and morphology [30].Therefore the interactions of silver nanoparticles with microbes rely on their definite shape and size [31]. Silver ions are extremely reactive and is easily ionized when exposed to moist environment .Therefore silver ions strongly binds to bacterial proteins and DNA causing disruption of its nuclear membrane , cell wall[32,33] and inhibition of bacterial replication leading to cell death [34,35].

ISSN 2348 – 8034
Impact Factor- 4.022

Generally speaking there are three mode of antimicrobial mechanism of silver nanoparticles [36] which are as follows:

1. Direct or indirect interactions of nanoparticles with the bacterial cell resulting in disruption of plasma membrane, DNA replication, and inhibition of respiratory protein causing lipid peroxidation etc
2. Penetration of the nanoparticles across the microbial membrane thus inhibiting oxidation [37].
3. Binding of silver nanoparticles to functional groups in microbes exhibiting oligodynamics resulting in the precipitation and inactivation of bacterial cell [38].

Therefore enhanced antimicrobial spectrum of silver nanoparticle is dependent on two features its large surface area and small size. Large surface area provides desirable contact with the bacterial cell and increase specificity while the smaller size enables easy penetration into bacteria through the cell wall [39, 40]. For example silver nanoparticles produced from endophytic fungi *Pestalotia sp* have shown broad antibacterial spectrum against S. *aureus* and *S. typhi* [41].

2. Anti-viral effects

Antiviral properties of silver nano crystals have been demonstrated by the binding of Silver nanoparticles with the glycoprotein present in the viral envelope thus inhibiting the infectivity of *HIV-1* virus [42] .These nanoparticles having size range between 1-10 nm.

3. Anti-fungal effects

Silver nanoparticles from *A. alternata* possess antifungal activity against *P. glomerata*, *Trichoderma sp* and *C. albicans.*When used in combination with fluconazole showed increased activity against *Candida albicans* followed by *Trichoderma sp* and *Phoma glomerata*[21].In another study role of Silver nanoparticles in growth inhibition of *M. oryzae*[28] and *C. albicans* [29] have also been well documented.

4. Vector control

Silver nanoparticles from entomopathogenic fungus *Beauveria bassiana* were effective against dengue vector *Aedes aegypti*[43]. Similarly the effect of Silver nanoparticleswas evaluated against filiariasis vector *Culex quinquefasciatus* which was produced from *Chrysosporium keratinophilium, Verticillium lecanii* and *F. oxysporum*[44].

5. Wound healing

Silver nanocrystalsproduced from *Aspergillus niger*havewound healing property [45] explained with the help of two proposed models i.e. Excision woundmodel and thermal wound model. It suggested Silver nanoparticles as better wound healing property as demonstrated by measuring degree of wound contraction and period of epithelialization in time and dose dependent manner.While *Phytophthora infestans* synthesized Silver nanoparticles was found to have 0.125% (w/w) Silver nanoparticles ointments having better wound healing property in contrast to standard silver sulphadiazine ointment [46].

6. Food industry

Incorporation of *T. viride* synthesized silver nanoparticle into sodium alginate thin film showed antibacterial potency and increases the shelf life of pear and carrot[47]

7. Chemical industry

Silver nanoparticles have diverse applications in dentistry, textiles, catalysis, optics, photography, and electronics which has tremendously increased its market value [24]. Silver nanoparticles have been used in the manufacture of electrical batteries, staining pigments and polarizing filters etc [48]

[Khan, 4(3): March 2017] ISSN 2348 – 8034
DOI-10.5281/zenodo.438942 Impact Factor- 4.022

8. Textile industry

Amalgamation of *F. oxysporum* fabricated Silver nanoparticles into cotton fabrics inhibits *S. aureus*[49].On the other hand *Lecanicillium lecanii* mediated silver nanoparticles when incorporated within cotton fabrics has shown to inhibit the growth of *S. aureus* and *E. coli*[50] thus making it suitable antibacterial agents.

9. Molecular Detection

Conjugation of silver nanoparticles with master mix and DNA sample of *Candida sp*have been used for rapid detection of pathogenic fungi .This silver bioconjugate nano PCR assay have shown high specificity and sensitivity as compared to conventional method [51].

V. CONCLUSION

In the light of above mentioned examples, we therefore concluded that nitrate reductase mediated silver nanoparticle synthesis is reported in a number fungal species producing nanoparticles that possess unique properties. Hence their uniqueness is the main reason that these nanoparticles are employed in several applications

REFERENCES

1. *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 AuCl4 ions by the fungus Verticillium sp and surface trapping of the gold nanoparticles formed. Angewante Chemie International Edition 40(19): 3585-3588.*
2. *Sastry M, Ahmad A, Khan MI, Kumar R.(2003) Biosynthesis of metal nanoparticles using fungi and Actinomycetes. Curr Sci 85: 162-70.*
3. *Vijayaraj D, Anarkali J, Rajathi K, and Sridhar S. (2012) Green synthesis and characterization of silver nanoparticles from the leaf extract of aristolochia bracteata and its antimicrobial efficacy. International Journal of Nanomaterials and Biostructures vol. 2, pp. 11–15.*
4. *Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M. (2003) Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. Colloids Surf, B 28: 313-318.*
5. *Durán N, Marcato PD, Alves OL, de Souza GIH, Esposito E. (2005) Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains. J Nanobiotechnology 3: 1-8.*
6. *Kashyap PL, Kumar S, Srivastava AK, Sharma AK. (2013) Myconanotechnology in agriculture: a perspective. World J Microbiol Biotechnol 29(2): 191-207.*
7. *Basavaraja S, Balaji SD, Lagashetty A, Rajasab AH, Venkataraman A.(2007) Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium semitectum. Material Research Bulletin 43(5): 1164-1170.*
8. *Gade A, Bonde PP, Ingle AP, Marcato P, Duran N, Rai MK.(2008) Exploitation of Aspergillus niger for synthesis of silver nanoparticles. Journal of Bio based Materials and Bioenergy 2(3): 1–5.*
9. *Kumar AS, Ansary AA, Ahmad A, Khan MI.(2007a) Extracellular Biosynthesis of CdSe Quantum Dots by the Fungus Fusarium oxysporum. Journal of Biomedical Nanotechnology 3: 190-194*
10. *Sangiliyandi G et al. (2009) Biosynthesis, Purification and Characterization of Silver Nanoparticles using Escherichia coli. Colloids and Surfaces B: Biointerfaces Vol.74, pp. 328-335*
11. *Srivastava P, Bragança J, Ramanan SR, Kowshik M. (2013) Synthesis of silver nanoparticles using haloarchaeal isolate Halococcus salifodinae BK3. Extremophiles 17(5): 821-831.*
12. *Devi TP, Kulanthaivel S, Kamil D, Borah JL, Prabhakaran N, Srinivasa N. (2013) Biosynthesis of silver nanoparticles from Trichoderma species. Indian J Exp Biol 51(7): 543-547.*
13. *Manivasagan P, Venkatesan J, Senthilkumar K, Sivakumar K, Kim SK.(2013) Biosynthesis, antimicrobial and cytotoxic effect of silver nanoparticles using a novel Nocardiopsis sp. MBRC-1. Biomed Res Int 287638.*

ISSN 2348 – 8034
Impact Factor- 4.022

14. Hamedi S, Shojaosadati SA, Shokrollahzadeh S, Hashemi-Najafabadi S. (2014) Extracellular biosynthesis of silver nanoparticles using a novel and non-pathogenic fungus, Neurospora intermedia: controlled synthesis and antibacterial activity. World J Microbiol Biotechnol 30(2): 693-704.
15. Gholami-Shabani M, Akbarzadeh A, Norouzian D, Amini A, Gholami-Shabani Z, Imani A, Chiani M, Riazi G, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M.(2014) Antimicrobial activity and physical characterization of silver nanoparticles green synthesized using nitrate reductase from F. oxysporum. Appl Biochem Biotechnol 172(8): 4084-4098.
16. Ingle A, Gade A, Pierrat S, So¨nnichsen C, Rai M.(2008) Mycosynthesis of silver nanoparticles using the fungus F. acuminatum and its activity against some human pathogenic bacteria. Curr Nanosci 4(2): 141–144.
17. Austin LA, Mackey MA, Dreaden EC, El-Sayed MA. Archives of toxicology 88(7), 1391-1417.
18. McConnell WP, Novak JP, Brousseau LC, Fuierer RR, Tenent RC, Feldheim DL. (2000) Journal of Physical Chemistry B 104(38), 8925-8930.
19. Majdalawieh A, Kanan MC, El-Kadri O, Kanan SM.(2014) Journal of nanoscience and nanotechnology 14(7), 4757-4780.
20. Prathna T, Raichur AM, Chandrasekaran N, Mukherjee A. (2014) Reviews in Advanced Sciences and Engineering, 3(3), 239-249.
21. 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. Nanomed Nanotechnol Biol Med 5(4): 382–386.
22. 22. Qian Y, Yu H, He D, Yang H, Wang W, Wan X, Wang L.(2013) Biosynthesis of silver nanoparticles by the endophytic fungus Epicoccum nigrum and their activity against pathogenic fungi. Bioprocess and Biosystems Engineering 36(11): 1613-1619.
23. Panacek A, Kvítek L, Prucek R, Kolar M, Vecerova R, Pizúrova N, Sharma VK, Nevecna T, Zboril R. (2006) Journal of Physical Chemistry B 110, 16248–16253.
24. Ghaffari-Moghaddam M, Hadi-Dabanlou R, (2014) Journal of Industrial and Engineering Chemistry 20(2), 739-744.
25. Saravanan M, Nanda A. (2010) Extracellular synthesis of silver bionanoparticles from Aspergillus clavatus and its antimicrobial activity against MRSA and MRSE. Colloids Surf. B Biointerfaces 77, 214–218.
26. Nanda A, Saravanan M. (2009) Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE. Nanomedicine 5(4):452–456.
27. Afreen RV and Ranganath E. (2011) Synthesis of monodispersed silver nanoparticles by Rhizopus stolonifer and its antibacterial activity against MDR strains of Pseudomonas aeruginosa from burnt patients. Int J Environ Sci 1(7): 1582–1592.
28. Ray S, Sarkar S, Kundu S. (2011) Extracellular biosynthesis of silver nanoparticles using the mycorrhizal mushroom Tricholoma crassum (Berk.) Sacc: its antimicrobial activity against pathogenic bacteria and fungus, including multidrug resistant plant and human bacteria. Dig J Nanomater Bios 6(3): 1289-1299.
29. Verma VC, Kharwar RN, Gange AC. (2010) Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus Aspergillus clavatus. Nanomedicine 5(1): 33–40.
30. Nithya R, Ragunathan R. (2009) Synthesis of silver nanoparticle using Pleurotus sajor caju and its antimicrobial study. Dig J Nanomater Bios 4(4): 623–629.
31. Sathyavathi R, Krishna MB, Rao SV, Saritha R, Rao DN. (2010) Biosynthesis of silver nanoparticles using Coriandrum sativum leaf extract and their application in nonlinear optics. Adv Sci Lett 3(2):138–143.
32. Savithramma N, Rao ML, Rukmani K and Suvarnalathadevi P.(2011)Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. International Journal of Chemical Technology Research 3: 1394–1402.

33. *Rajathi K and Sridhar S (2013) Green synthesized silver nanoparticles from the medicinal plant Wrightia twictoria and its antimicrobial potential. International Journal of Chemical Technology Research 5:1707–1713.*
34. *Costellano JJ, Shafii SM, Ko F, Donate G, Wright TE, Mannari RJ, Payne WG, Smith DJ and Robson MC .(2007) Comparative evaluation of silver-containing antimicrobial dressings and drugs, International Wound Journal 4 :114–122.*
35. *Chadeau E, Brunon C, Degraeve P, Leonard D, Grossinord C, Bessueille F, Cottaz A, Renaud F, Ferreira I, Darroux C, Simon F, Rimhault F and Oulahal N.(2012) Evaluation of antimicrobial activity of a polyhexamethylene biguanide-coated textile by monitoring both bacterial growth and viability (live/dead baclight kit). Food Safety 32:141–151.*
36. *Liu JI, Luo Z and Bashir S. (2013) A progressive approach on inactivation of bacteria using silver-titania nanoparticles, Biomaterial Sciences 1:194–201*
37. *Sereemaspun A, Hongpiticharoen Rojanathanes Maneewattanapinyo P, Ekgasit Sand Warisnoicharoen W. (2008) Inhibition of human cytochrome P450 enzymes by metallic nanoparticles: A preliminary to nanogenomics. International Journal of Pharmacology 4: 492–495.*
38. *Parashar V, Parashar R, Sharma B and Pandey AC. (2009) Parthenium leaf extract mediated synthesis of silver nanoparticles: A novel approach towards weed utilization, Digest Journal of Nanomaterials and Biostructures 4:45–50.*
39. *Martinez-Gurierrer F, Thi EP, Silverman J, de Oliveira CC, Svensson SL, Hock AV, Sancher EM, Reiner NE, Gaynor EC, Pryzdial ELG, Conway EM, Orrantia E, Ruiz F, Av-Gay Y and Bach H.(2012) Antibacterial activity, inflammatory response, coagulation and cytotoxicity effects of silver nanoparticles, Nanomedicine 8 : 328–336.*
40. *Barani H, Montazer M, Samadi N and Toliyal T. (2012) In situ synthesis of nano silver/ lecithin on wool: Enhancing nanoparticles diffusion, Colloids and Surfaces B: Biointerfaces 92 :9–15.*
41. *Raheman F, Deshmukh S, Ingle A, and Gade A, Rai M.(2011) Silver nanoparticles: novel antimicrobial agent synthesized from an endophytic fungus Pestalotia sp. isolated from leaves of Syzygium cumini (L). Nano Biomed Eng 3(3):174–178.*
42. *Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH, Yacaman MJ.(2005) Interaction of silver nanoparticles with HIV-1. Journal of Nanobiotechnology 3 (6): doi: 101186/1477-3155-3-6.*
43. *Banu NA, Balasubramanian C. (2014) Myco-synthesis of silver nanoparticles using Beauveria bassiana against dengue vector, Aedes aegypti (Diptera: Culicidae). Parasitology Research 113(8); 2869-2877.*
44. *Soni N, Prakash S. (2012) Fungal-mediated nano silver: an effective adulticide against mosquito. Parasitol Res 111(5): 2091–2098.*
45. *Sundaramoorthi C, Kalaivani M, Mathews DM, Palanisamy S, Kalaiselvan V, Rajasekaran A.(2009) Biosynthesis of silver nanoparticles from Aspergillus niger and evaluation of its wound healing activity in experimental rat model. International Journal of Pharm Tech Research 1(4): 1523-1529.*
46. *Thirumurugan G, Veni VS, Ramachandran S, Rao JV, Dhanaraju MD. (2011) Superior wound healing effect of topically delivered silver nanoparticle formulation using eco-friendly potato plant pathogenic fungus: synthesis and characterization. J Biomed Nanotechnol 5: 659-666.*
47. *Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R. (2009) Mycobased synthesis of silver nanoparticles and their incorporation into sodium alginate films for vegetable and fruit preservation. J. Agric. Food Chem 57, 6246–6252.*
48. *Maliszewska I, Szewczyk K, Waszak K. (2009) Biological synthesis of silver nanoparticles. J. Phys. Conf. Ser 146, doi:10.1088/1742-6596/146/1/012025.*
49. *Duran N, Marcato PD, De Souza GIH, Alves OL, Esposito E.(2007) Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. Journal of Biomedical Nanotechnology 3(2): 203–208.*
50. *Namasivayam SKR, Avimanyu. (2011) Silver nanoparticle synthesis from lecanicillium lecanii and evalutionary treatment on cotton fabrics by measuring their improved antibacterial activity with*

[Khan, 4(3): March 2017]
DOI-10.5281/zenodo.438942
ISSN 2348 – 8034
Impact Factor- 4.022

antibiotics against Staphylococcus aureus (ATCC 29213) and E coli (ATCC 25922) strains. International Journal of Pharmacy and Pharmaceutical Sciences 3(4): 190-195.

51. *Bansod S, Bonde S, Tiwari V, Bawaskar M, Deshmukh S, Gaikwad S, Gade A, Rai M.(2013) Bioconjugation of gold and silver nanoparticles synthesized by F. oxysporum and their use in rapid identification of Candida species by using bioconjugate-nano-polymerase chain reaction. J Biomed Nanotechnol 9(12): 1962-1971*