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OPTIMIZATION OF MYCOBIOSYNTHESIS OF SILVER NANOPARTICLES BY USING *FUSARIUM* 4F1 AND *TRICHODERMA* TRS ISOLATES

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Abstract

Silver nanoparticles (Ag-NPs) by mixing silver nitrate ($AgNO_3$) with cell-free filtrate (CFF) of the two fungal isolates *viz.*, *Fusarium* 4F1 and *Trichoderma* TrS were synthesized. pH, substrate concentration and incubation period for the production of better quality and quantity of Ag-NPs was optimized. The Ag-NPs by UV-vis spectroscopy were characterized. Between the two fungal isolates, pH levels, $AgNO_3$ concentrations and incubation periods studied, the highest number of spherical shaped, monodispersed and stable Ag-NPs were recorded from *Fusarium* 4F1 at pH 9, 2 mM $AgNO_3$ and 72 hrs of incubation.

Introduction

Nanoparticles (NPs) are regarded as highly reactive elements because of the large surface area. They have novel magnetic, electronic and optical properties, which vary on the basis of their size, shape, and composition (Patil 2014). At present, different types of metal NPs are being produced. They have a wide range of applications in areas such as health care, biomedical sciences, cosmetics, drug-gene delivery, bactericidal, biological labeling, treatment of some cancers, optics, chemical industries, electronics, space industries, single electron transistors, light emitters, sensor technology, etc. (Iravani *et al*. 2014).

Numerous protocols have been developed to synthesize NPs following chemical, physical and biological methods (Rai *et al*. 2009). Among them, biological methods are considered as eco-friendly, high yielding, reliable, clean, and nontoxic (Gupta *et al*. 2012, Iravani *et al*. 2014, Patil 2014). Biosynthesis would have greater commercial acceptance if the NPs could be synthesized more rapidly and economically on a large scale. However, in order to achieve better control of size, morphology, stability, and NP production rate, biological methods could be used with some optimization (Korbekandi *et al*. 2013).

Microbes have been explored as a potential bio-factory for the synthesis of different metallic NPs (Korbekandi *et al*. 2013). Among different microbes some fungal isolates are capable of the NPs formation. The fabrication of NPs by fungi is an exciting modern field of applied interdisciplinary science. Because fungi have considerable potential in biosynthesis of NPs owing to their wide range and diversity (Rai *et al*. 2009).

Among different metal NPs, silver nanoparticles (Ag-NPs) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts. Ag-NPs play a significant role in the field of biology and medicine (Patil 2014). Ag-NPs can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, water purification systems, and electronic components. A combination of antibiotics and metal Ag-NPs could increase the antibiotics' efficacy against resistant pathogens (Fayaz *et al*. 2010, Sheng and Liu 2011).

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Mycobiosynthesis of Ag-NPs has not been reported so far in Bangladesh. The aim of the present work was to synthesize Ag-NPs in laboratory scale by Bangladeshi fungal isolates obtained from soil and optimized pH level, substrate concentration and incubation period for the production of higher quality and quantity of Ag-NPs.

Materials and Methods

Soil samples were collected from the pine plantation of the National Botanical Garden, Mirpur, Dhaka, Bangladesh. Fungi were isolated in pure culture following serial dilution and pour plate method using potato dextrose agar (PDA) medium supplemented with 0.1 mg/ml streptomycin sulfate to exclude soil bacteria (Aneja 2003). The fungal isolates were identified following standard literatures on the basis of their morphological characteristics (Booth 1971, Bissett 1991). Among the isolated fungi, *Fusarium* 4F1 and *Trichoderma* TrS were selected for the biosynthesis of Ag-NPs because the genera are reported to be promising Ag-NPs producer (Vahabi *et al*. 2011, Birla *et al*. 2013, Korbekandi *et al*. 2013).

To prepare biomass, *Fusarium* 4F1 and *Trichoderma* TrS were grown aerobically in potato sucrose and potato dextrose broth media (pH 7), respectively at 24 ± 2°C. The culture broth in the flasks were agitated at 170 rpm on a rotary shaker for 72 hrs. The biomass was harvested after incubation using a plastic sieve. After that, it was thoroughly washed with sterile distilled water to remove any other component from the biomass.

To prepare Cell-Free Filtrate (CFF), approximately 20 g of fresh and clean biomass were taken in a 500 ml Erlenmeyer flask containing 150 ml of sterilized deionized water. The flask was incubated at 24 ± 2°C on a rotary shaker for 72 hrs and agitated at 170 rpm. After the incubation period, CFF was obtained by passing it through sterile Whatman filter paper No. 1.

For the biosynthesis of Ag-NPs, the silver nitrate ($AgNO_3$) solution was mixed with *ca.* 50 ml fresh CFF of *Fusarium* 4F1 and *Trichoderma* TrS in several sets in 250 ml Erlenmeyer flasks. The final concentration of the substrate ($AgNO_3$) in the solutions was 1 mM and pH was adjusted to 7, 8, and 9 separately. The flasks containing CFF without $AgNO_3$ at pH 7, 8 and 9 were also maintained along with the treatment sets. These flasks were marked as control. Depending on the effects of pH on the quality and quantity of Ag-NPs, separate sets of experiments were run where final concentration of the $AgNO_3$ were 2 mM and pH were 9 for both fungal isolates following the above mentioned procedure. In every sets, 3 replications were maintained. All flasks were fully covered with thick black paper and incubated on a rotary shaker and agitated at 170 rpm at 24 ± 2°C in dark.

Flasks were visually examined after every 24 hrs intervals up to 45 days to find any kind of change of CFF and $AgNO_3$ mixture color. In every observation, data on color change were recorded and photographs of the flasks were also taken with a digital camera. Reaction mixture was withdrawn every after 24, 48 and 72 hrs from each flask. UV-visible light absorption profile of the mixture was recorded to study the change in intensity of the brown color during incubation periods using a UV-Vis spectrophotometer (SPECORD 250 plus, Analytic Jena). The absorbance was measured from 300 - 600 nm at a resolution of 1 nm. The control sets were used as reference in spectroscopy. It is generally recognized that UV-Vis spectroscopy could be used to examine the quality and quantity of NPs in aqueous suspensions (Wiley *et al*. 2006). The results were analyzed statistically for the test of significance using WASP 2.0 software. The experiments were conducted in the laboratory at the department of Botany, Jagannath University, Dhaka.

Results and Discussion

The treatment flasks containing reaction mixture showed a change in color from almost colorless or light yellow to light brown or brown after 24, 48 or 72 hrs of incubation. The control sets did not show any change of color (Fig. 1). The appearance of the brown color was an indication of the formation of Ag-NPs in the reaction mixture. This observation was in agreement with the previous reports, where the brown color was considered as the production of colloidal suspension (hydrosol) of Ag-NPs (Ingle *et al*. 2008, Korbekandi *et al*. 2013). Metal NPs exhibited different colors in solution due to their optical properties. The appearance of brown color was owing to the excitation of free electrons in NPs. It gives the Surface Plasmon Resonance (SPR) absorption band by the combined vibration of electrons of metal NPs in resonance with light waves (Rai *et al*. 2009).

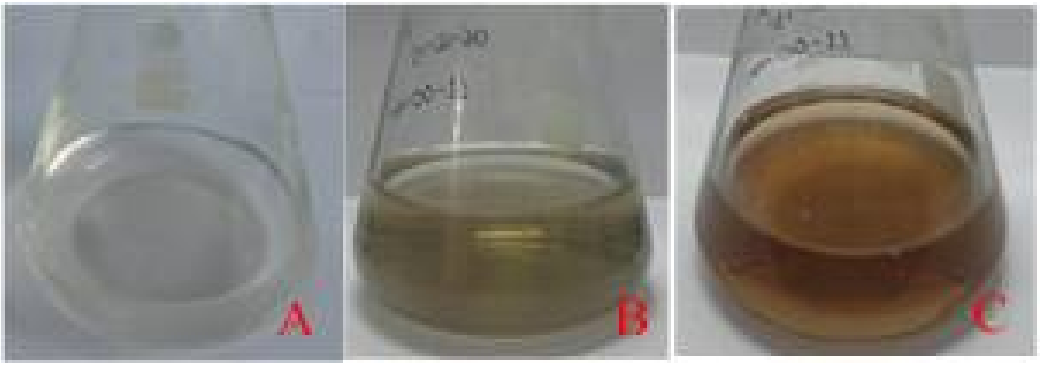

Fig. 1. Mycobiosynthesis of silver nanoparticles (Ag-NPs). A. Cell free filtrate (CFF) of *Fusarium* sp. B. CFF treated with 1 mM $AgNO_3$ (final concentration) at pH 9. C. Color intensified at the treatment flask after 24 hrs incubation.

Ag-NPs are synthesized into the reaction mixture by the reduction of silver ions (Ag^+) to metallic silver (Ag^0). The silver ions may require the NADH and NADH-dependent nitrate reductase enzyme for their reduction. Fungi may release proteins/enzymes into the extracellular CCF. The reduction may occur due to the transfer of an electron from NADH where the NADH-dependent reductase can act as an electron carrier. Consequently, the proteins also may bind to the NPs and enhance the stability (Duran *et al*. 2005).

At pH 7 and 8, the color intensity of the reaction mixture gradually increased up to 9 - 10 days after incubation. It turned brown to deep blackish brown and remained constant after 45 days of observation for both the fungal isolates. A small amount of sedimentation also occurred at the reaction flasks (Table 1). The increase of color intensity could be owing to the gradual increasing of Ag-NPs and/or their aggregation. Sedimentation and turn of color into black/dark after a long-standing at pH 7 and 8 was owing to much aggregation of Ag-NPs and/or conversion of Ag-NPs into silver oxide (Ag_2O). The color of the aggregated Ag-NPs, became black because of its large size and losing of the property of SPR (Ingle *et al*. 2008, Rai *et al*. 2009).

At pH 9, the color intensity and light absorbance increased up to 72 hrs and remained constant after 45 days observation. There was no sedimentation in the reaction flask (Table 1 and Fig. 1). It might be due to the absence of aggregation of Ag-NPs in reaction mixture at pH 9 for both the fungal isolates. It indicates that Ag-NPs were relatively stable at pH 9. It might be due to the adsorption of OH^- on Ag-NPs, while at lower pH aggregates were formed due to the unavailability

of OH^- ions (Birla *et al*. 2013). Again high stability of NPs in the solution was found due to capping of the particles with certain proteins present in CFF which were released by the fungi. Stability of capping proteins depends on pH. At higher pH values, the NPs in the solution remained stable, while they are aggregated at lower pH values as the protein was denatured (Kumar *et al*. 2007).

Table 1. UV-Vis spectroscopic properties of the reaction mixture containing CFF of *Fusarium* 4F1 and *Trichoderma* TrS at different pH levels, incubation periods and substrate concentrations.

Fungal isolate name, substrate conc. and pH level	Incubation period						Presence of sediment
	24 hrs		48 hrs		72 hrs		
	WAP (nm)*	Absorbance (a.u.)	WAP (nm) *	Absorbance (a.u.)	WAP (nm)*	Absorbance (a.u.)	
Fusarium 4F1:							
At 1 mM $AgNO_3$ final concentration							
pH 7	428	0.0195^g	436	0.0203^h	442	0.0211^f	Scanty
pH 8	448	0.0211^g	457	0.0232^g	467	0.0261^f	Scanty
pH 9	440	0.3126^a	440	0.3345^b	440	0.3690^b	No
At 2 mM $AgNO_3$ final concentration							
pH 9	425	0.2905^c	425	0.3801^a	425	0.4556^a	No
Trichoderma TrS:							
At 1 mM $AgNO_3$ final concentration							
pH 7	443	0.0841^f	442	0.1153^f	441	0.1489^e	Scanty
pH 8	455	0.1183^e	456	0.1402^e	456	0.1616^d	Scanty
pH 9	438	0.2831^d	439	0.2943^d	439	0.3306^c	No
At 2 mM $AgNO_3$ final concentration							
pH 9	428	0.3055^b	429	3267^c	429	3378^c	No
CD (0.01)		0.001		0.006		0.001	
CD (0.05)		0.004		0.001		0.008	
CV**		1.427		0.322		0.198	

*WAP = Average wavelength of absorbance peak. ** All treatments found significant at 1 and 5% level of significance. Same letter beside means of absorbance in a column represent insignificant difference among them at 5% level.

The UV-Vis spectra at absorbance maxima of the reaction mixture containing CFF of *Fusarium* 4F1 and 1 mM $AgNO_3$ solution at pH 7, 8 and 9 for 24, 48 and 72 hrs of incubation period are presented in the Table 1. At pH 7, a strong absorbance peak was exhibited at 428 nm wavelength after 24 hrs of incubation and shifted to 436 and 442 nm with increasing absorbance value as the reaction proceeded up to 48 and 72 hrs of incubation, respectively. The same trends were observed at pH 8. Whereas at pH 9 the spectrum was stable at 440 nm but the absorbance was increased gradually up to 72 hrs of incubation periods. The spectra at absorbance maxima were stable at 425 nm for 2 mM $AgNO_3$ solution and gradual increase of the absorbance was also recorded. The highest absorbance value (0.4556 a.u.) was recorded here after 72 hrs of incubation.

In the case of *Trichoderma* Trs isolate, little (± 1) or no shifting of spectra was found in all the reaction mixture sets. Gradual increase of the absorbance was also recorded. The highest absorbance (0.3378 a.u.) was recorded at 429 nm, pH 9 and 2 mM $AgNO_3$ solution.

Close relationship between the UV-Vis absorbance peak and size and shape of Ag-NPs was detected by former workers (Sosa *et al*. 2003). The shift of spectrum towards smaller or longer wavelengths depends upon the particle size, shape and state of aggregation (Park and Kim 2008). According to the Mie's theory, only a single SPR band is expected in the absorption spectra of spherical metal NPs. Spherical Ag-NPs showed specific absorbance peak around 420 (± 10) nm (Novak and Feldheim 2000). At the same UV-Vis spectrum, an increase of absorption indicates increased amount of Ag-NPs at colloidal suspension. The stable position of absorbance peak indicates that the NPs do not aggregate (Vahabi *et al*. 2011).

From this study, it may be inferred that pH 9, 72 hrs of incubation period and 2 mM concentration of $AgNO_3$ are optima for mycobiofabrication of spherical, monodispersed and stable Ag-NPs by both fungal isolates.

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References

Aneja KR 2003. Experiments in Microbiology, Plant Pathology and Biotechnology. 4th ed. New Age International, New Delhi, India. pp. 607.

Birla SS, Gaikwad SC, Gade AK and Rai MK 2013. Rapid synthesis of silver nanoparticles from *Fusarium oxysporum* by optimizing physicocultural conditions. Sci. World J. **2013**: 1-12.

Bissett J 1991. A revision of the genus *Trichoderma*. III. Section *Pachybasium.* Canad. J. Bot. **69**(11): 2373-2417.

Booth C 1971. The genus *Fusarium.* Commonwealth Mycological Institute, Kew, Surrey, England. pp. 237.

Duran N, Marcato PD, Alves OL, De Souza IH and Esposito E 2005. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. J. Nanotech. **3**(8): 1-7.

Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT and Venketesan R 2010. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. Nanomedi. **6**(1): 103-109.

Gupta S, Sharma K and Sharma R 2012. Myconanotechnology and application of nanoparticles in biology. Recent Res. in Sci. and Tech. **4**(8): 36-38.

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

Iravani S, Korbekandi H, Mirmohammadi SV and Zolfaghari B 2014. Synthesis of silver nanoparticles: chemical, physical and biological methods. Res. in Pharmaceutical Sci. **9**(6): 385-406.

Korbekandi H, Ashari Z, IravaniS and Abbasi S 2013. Optimization of biological synthesis of silver nanoparticles using *Fusarium oxysporum.* Iranian J. Pharmaceutical Res. **12**(3): 289-298.

Kumar AS, Abyaneh MK, Sulabha SWG, Ahmad A and Khan MI 2007. Nitrate reductase-mediated synthesis of silver nanoparticles from $AgNO_3$. Biotechnol. Lett. **29**(3): 439-455.

Novak JP and Feldheim DL 2000. Assembly of phenylacetylene-bridged gold and silver nanoparticle arrays. J. Am. Chem. Soc. **122**(16): 3979-3980.

Park J and Kim Y 2008. Effect of shape of silver nanoplates on the enhancement of surface plasmon resonance (SPR) signals. J. Nanosci. Nanotech. **8**: 1-4.

Patil SR 2014. Antibacterial activity of silver nanoparticles synthesized from *Fusarium semitectum.* CIB Tech. J. Biotech. **3**(1): 08-12.

Rai M, Yadav A, Bridge P and Gade A 2009. Myconanotechnology: a new and emerging science. *In*: Rai M. and Bridge P (Eds.) Applied Mycology, pp. 258-267. CAB International, Oxfordshire, UK.

Sheng Z and Liu Y 2011. Effects of silver nanoparticles on wastewater biofilms. Water Res. **45**(18): 6039-6050.

Sosa IO, Noguez C and Barrera RG 2003. Optical properties of metal nanoparticles with arbitrary shapes. J. Phys. Chem. B. **107**(26): 6269-6275.

Vahabi K, Mansoori GA and Karimi S 2011. Biosynthesis of silver nanoparticles by fungus *Trichoderma reesei* (a route for large-scale production of AgNPs). Insciences J. **1**(1): 65-9.

Wiley BJ, Im SH, Li ZY, McLella J, Siekkinen A and Xia Y 2006. Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. J. Phys. Chem. B. **110**: 15666-15675.

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