International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.7, No.01, pp 68-72, 2014-2015

Silver nanoparticles synthesized from marine fungi *Aspergillus oryzae*

B. Valentin Bhimba[1], Smriti Gurung[1,2], S. Usha Nandhini

[1]Department of Biotechnology, Sathyabama University, Chennai, India
[1,2]School of Medicine, Kyungpook National University, South Korea

Abstract: In the present study six fungal strains were isolated from marine mangrove sediment from parangipettai on Saborauds agar. The pure culture was grown in Saborauds broth at 27^0 c for 5 days in a shaker incubator. After incubation, the mycelium was separated and washed twice with milli Q water. 20 g of biomass was treated with 200ml of milliQ water for 72 hours at 27^0 c and agitated in the shaker incubator. After incubation, the cell was filtered through Whatmann filter paper and the 1mM silver nitrate solution was mixed with 50 ml of cell filtrate and kept at 25^0c in dark. Control was prepared without addition of Silver nitrate solution. The nanoparticles synthesized showed an absorption peak at 420nm in UV-Vis spectrum corresponding to the Plasmon resonance of silver nanoparticles, thus, confirming their presence. The Fourier transform infrared spectroscopy confirmed the presence of protein as stabilizing agent surrounding the silver nanoparticles. This elite fungal strain was identified and confirmed as *Aspergillus oryzae* by isolating their genomic DNA and amplifying the ITS region present in 5.8s rRNA and sequenced using ITS primer1 and 4.
Key words Silver nanoparticles, Aspergillus oryzae, marine fungi.

Introduction

Marine derived fungi are a rich source of structurally new natural products with a wide range of biological activities[1-4]. Natural products research is turned for marine animals and plants. A lot of structurally and pharmacological new and interesting substances have been, for instance, isolated from algae. Compared to these organisms, marine fungi are poorly investigated. The associateion between algae and fungi appears to be highly developed since nearly one third of all higher marine fungi described are so called algicolous or algae associated organisms[5]. Consequently increasing attention has been paid to bioreduction in efficient and green chemistry approach. For silver bioreduction, examples include the fungus *verticillium* sp., and *Fusarium pxysporum* which are able to reduce the metal ions into silver nanaparticles intracellularly and extracellularly respectively.

Methodology

The fungal strains used were isolated from the mangrove sediment soil using Sabourauds Agar with the supplementation of 50 percent sterile seawater at 28^0C in petri plates. The liquid fungal growth was carried out in the presence of a yeast extract 0.5% at 28^0C in shaker incubator (150rpm) for 72 hrs. The biomass was extensively washed to remove the media component and filtrated (Whatman Filter paper). Fresh and clean biomass of around 20g was taken and resuspended in Milli Q water .The flasks were agitated at the same conditions as described above, then the biomass was filtered again (Whatman filter paper No. 1) after 5 days and cell-free filtrate was used for further studies.

Silver reduction

1mM $AgNO_3$ was added to the cell free filtrate and subjected to thermal treatment at 121˚C. The bio reduction of silver nitrate occurred within 10 min and a color change (dark brown) was noted by visual observation indicating the formation of AgNPs. As per the absorption spectrum, this medium remained stable for more than 3 months. Aliquots of the reaction solution were measured using a UV-1601 Schimadzu spectrophotometer operated at a resolution of 1nm.

Fourier transform infrared spectroscopy

The interaction between protein-silver nanoparticles were analyzed by Fourier transform infrared spectroscopy (FTIR) in the diffuse reflectance mode at a resolution of $4cm^{-1}$ in the KBr pellets and the spectra were recorded in the wavelength interval of 4000 to $400nm^{-1}$. FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the Ag+ ions and the capping of the bioreduced AgNPs. For comparison, the seaweed filtrate was mixed with KBr powder and pelletized after drying properly and subjected to measurement.

X-ray diffractometry

X-ray diffraction (XRD) measurement of the seaweed reduced AgNPs was carried out using powder X-ray diffractometer instrument (PXRD-6000 SCHIMADZU) in the angle range of 10˚C-80˚C at 2θ, scan axis: 2:1 sym. The size of the AgNPs was calculated from the PXRD peak positions using Bragg's law.

Transmission Electron Microscopy

This technique was employed to visualize the size and shape of AgNPs. Samples were prepared by drop coating AgNPs solutions onto carbon coated copper TEM grids. The films were allowed to dry under lamp following which the extra solution was removed using a blotting paper. TEM measurements were performed using TECNAI 10, Philips instrument operated at an accelerating voltage of 120 KeV.

Extended Dispersive analysis X-ray Spectroscopy

The presence of elemental Silver was confirmed through EDS. Energy dispersive analysis X-ray spectrometer takes advantage of the photon nature of the light. In the X-ray range the energy of a single photon is just sufficient to produce a measurable pulse X ray; the output of an ultra low noise pre-amplifier connected to the low noise is a statistical measure of the corresponding quantum energy. A semiconductor material is used to detect the X-rays together with processing electronics to analyses the spectrum. The EDX observations were carried out in STIC, CUSAT, Kerala (JOEL Model JED-2300).

Results and Discussion

The study of biosynthesis of nanomaterials offers a valuable contribution to nanobiotechnology. The biosynthesis methods have been investigated as an alternative to chemical and physical ones. In this regard, *A. oryzae* has been isolated (Fig.1) and identified by ITS sequencing which proves to be an important biological component for extracellular biosynthesis of stable AgNPs. It is well known that silver nanoparticles exhibits reddish color in water, this color arises due to excitation of surface Plasmon vibrations in the metal nanoparticles[6] Fig 2. The biomass incubated with de-ionised water (positive control) retained its original color while the silver nitrate treated biomass turned dark red after few hours due to the formation of silver nano particles extracellularly. This shows that it was a fast process. In case of negative control (silver nitrate solution alone), no change in color was observed and by UV-Vis spectra and SEM. It is observed from the spectra that the surface plasmon resonance band of AgNPs occurs at 420nm[7] and this absorption steadily increases in intensity as a function of time reaction (Fig.3). *Aspergillus oryzae* proves to be an important biological component for extracellular biosynthesis of AgNPs. It was observed that the reduction of the Ag ions during the exposure of *A. oryazae* proves to be an important biological component for extracellular biosynthesis of stable AgNPs. It was observed that the reduction of the Ag ions during the exposure to fungi filtrate can be easily followed by visual observation and UV vis spectroscopy. the absorption spectrum of dark brown AgNPs showed a surface Plasmon absorption band with a maximum of about 400nm, a characteristic peak of AgNPs [8-9], indicating the presence of AgNPs in the solution.

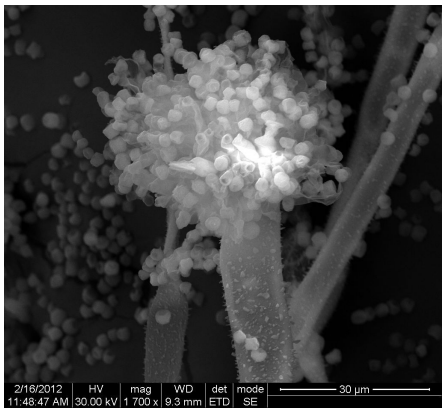


Fig 1. Electron Microscopic view of *Aspergillus oryzae*

Fig 2. Extracellular Ag Nanoparticles synthesized by *Aspergillus oruzae*

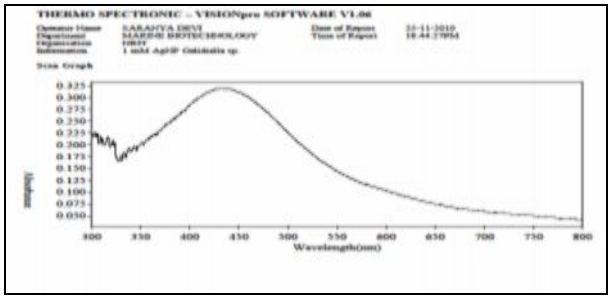


Fig. 3 UV-vis spectra recorded

It was observed from the FT-IR spectrum (Fig.4) of Ag NPs that the bands at 1637 correspond to a primary amine NH band. This evidence suggests that the release of extracellular protein molecules and possibly perform the function of the formation and stabilization of Ag NPs in aqueous medium. The fig shows the graph of the extract that does not contain $AgNO_3$ (control) and the extract containing $AgNO_3$ (sample). Spectra fig.8c shows the transmission peak at 3438.42, 2918.66, 2851.07, 2091.36, 1735.62, 1638.04, 1466.55, 1170.34, 1170.34, 1034.85, 721.89. Similarly transmission peak for the fungus *Aspergillus oryzae* containing silver nano particles were obtained at 3438.42, 2918.74, 2850.43, 2368.39, 2098.00, 1637.34, 721.02. Two absorption peaks located around 1637.34cm and 3439.46cm corresponds to Amide C=O and Amine groups respectively. Likewise 2918.74 corresponds to alkane C-H, 2850.43 to aldehyde C-H (2 peaks), 2368.39 to carboxylic acid O-H, 2098.00 to alkyne C=C, 1637.34 to amide C=O. Thus the result indicates that the amide, amine, alkane, aldehyde, carboxylic acid, alkyne groups of *Aspergillus oryzae* are mainly involved in the fabrication of silver nano particles.

XRD pattern of silver nano particles is shown in the Fig.5, whose peaks match with JCPDF card no. 087-0720. It exhibits a sharp and intense peak at 25 and 35. corresponds to diffraction from (111) and (101) plates of silver with fcc lattice JCPDS no. 04-0783.The peak plane matches with the card. The crystalline size is

calculated from the full width at half–maximum (FWHM) of the diffraction peaks using the Debye-Sherrer formula.

$$D = \frac{0.891\lambda}{\beta\cos\theta}$$

Where D means grain size is the x-ray wavelength, β is the FWHM of diffraction peak and 0 is the diffraction angle. The crystal size of the Ag nano particles in 4nm.

It is observed from TEM micrographs (Fig6) that most of the Ag NPs spherical and are in the range of 6-37nm in size. The TEM micrograph shows that the particles are polydispersed and are mostly spherical. Hence it can be understood that optimization of experimental conditions such as pH, temperature and concentration of Ag+ ions etc, will achieve mono dispersity and uniform shape. The typical HrTEM showing the size and morphology of monodisperse silver nano particles is given in the Fig.7. This picture shows individual silver particles as well as a number of aggregates. The morphology of the nano particles is variable with majority of them spherical. Under observation of such images were found to be aggregates of silver nano particles in the size range 6-37nm.

Fig 7 shows the SEM micrograph recorded from the silver nano particles. In this micrograph, spherical nano particles were observed. In the above SEM image, uniformly distributed silver nano particles on the surface of the cells were observed. However it does not indicate that all the nano particles are bound to the surface of the cells, because those disperging in the solution may also deposit onto the surface of the cell during the drying process which is a necessary step before SEM observation. The additional support of reduction of Ag+ ions to elemental silver, as confirmed by EDS analysis, shows a peak in the silver region, which confirms the presence of elemental silver (Fig.8). EDAX analysis gives quantitative status of elements that may be involved in the formation of nano particles in the given fig 8. It shows the EDAX (Energy Dispersive Analysis of X-Rays) spectrum recorded in the spot-profile mode from one of the densely populated silver nano particles region on the surface of the film.

The rapid biological synthesis of silver nanoparticles from fungi provides a simple and efficient route for the synthesis of nanoparticles with tunable optical properties directed by particle size. Future prospects of this research would be to scale-up the biosynthetic production of Silver nanoparticles and check its efficacy against a wide spectrum of Microbial population.

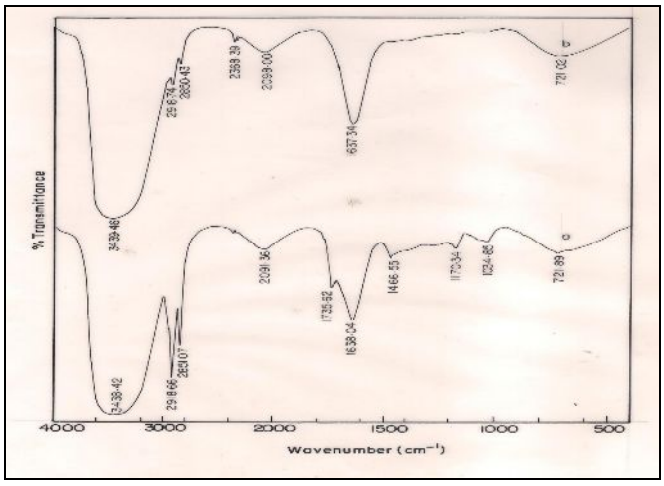


Fig. 4 Fluorescence emission spectrum recorded from the silver nanoparticles-fungus reaction mixture.

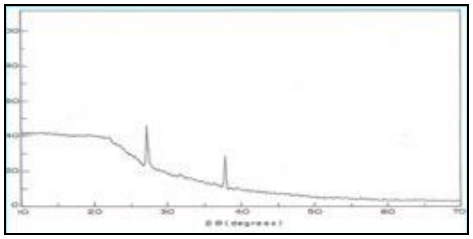


Fig 5. XRD pattern

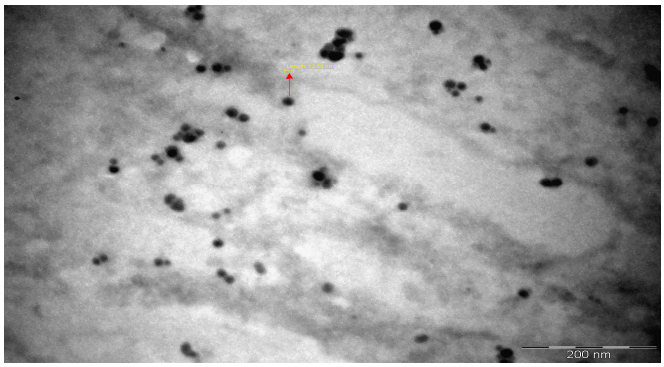
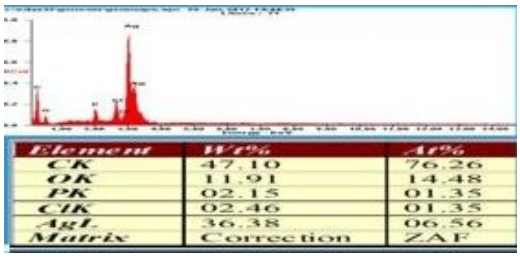


Fig 6. TEM micrograph of synthesized silver nanoparticles

Fig.7. SEM micrograph



Element	Wt%	At%
CK	47.10	76.26
OK	11.91	14.48
PK	02.15	01.35
ClK	02.46	01.35
AgL	36.38	06.56
Matrix	Correction	ZAF

Fig.8 EDAX

References

1. Smetanina, O.F.; Kalinovsky, A I.; Khudyakova, Y.V.; Pivkin, M.V.; Dmitrenok, P.S.; Fedorv, S.N.; Ji, H.; Kwak, J.Y. and Kuznetsova, T.A. Indole Alkaloids Produced by a Marine Fungus Isolate of Penicillium Janthinellum Biourge. Journal of Natural Products, 2007; 70: 906-909.
2. Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T. and Prinsep, M. R. Marine natural products. Natural Product Reports, 2006; 23: 26-78.
3. Pivkin, M. V.; Kuznetsova, T. A. and Sova, V. V. Marine fungi and their metabolites; Dalnauka: Vladivostok, 2006: 133.
4. Somei, M. and Yamada, F. Simple indole alkaloids and those with a non-rearranged monoterpenoid unit. Natural Product Reports, 2005; 22: 73-103.
5. Kohlmeyer, J.; Kohlmeyer, E.. In Marine Mycology, *The higher fungi, Academic press*; New York, San Francisco, London. 1979, p.132.
6. Henglein A. Physicochemical properties of small metal particles in solution: "microelectrode" reactions, chemisorption, composite metal particles, and the atom-to-metal transition. J. Phys. Chem., 1993; 97 (21): 5457–5471.
7. Ahmad A, Senapati S, Khan MI, kumar R, Srinivas V, Sastry M . Intracellular synthesis of silver nano particles by a novel alkalotolerant acitinomycete*, Rodococcus species*. Nanotechnology. 2003; 14: 824-828.
8. Petit C, Lixon P and Pileni M P. J. Phys. Chem. 1993; 97: 12974.
9. Kong and Jang, One-step fabrication of silver nanoparticle embedded polymer nanofibers by radical-mediated dispersion polymerization. Chem. Commun.2006; 3010-3012.

***** *****