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Biosynthesis Of Gold Nanoparticles By Marine Purple Non Sulphur Bacterium, Rhodopseudomonas Sp.

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Abstract: This paper describes for the first time that an anaerobic marine bacterium is capable of producing gold nanoparticles. A marine purple non-sulphur bacterium was isolated from mangrove sediment and identified as Rhodopseudomonas sp. . The bacterial culture was tested for the synthesis of gold nanoparticles by using aqueous HAuCl4 solution as substrate in darkness. The gold nanoparticles synthesized were found to be of cubical structure in the size range of 10–20 nm.

Keyword: Nanoparticles, Rhodopseudomonas sp.

INTRODUCTION:

Nanoscience is currently a fast growing niche and nanotechnology is at the cutting edge of this rapidly evolving area (Mandal et al., 2006). Nanotechnology collectively describes technology and science involving nano scale particles (nanoparticles) that increases the scope of investigating and regulating the interplay at cell level between synthetic materials and biological systems (Du et al., 2007). It can be employed as an efficient tool to explore the finest processes in biological processes (Sondi & Salopek-Sondi, 2004) and biomedical sciences (Hütten et al., 2004). Besides this, NPs play an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants and tissue engineering (Morones et al., 2005). In the current context, importance is being given to the fabrication of a wide range of nanomaterials for developing environmentally benign technologies in material synthesis (Bhattacharya et al., 2005).

Sizes and shapes of nanoparticles are of great importance for their applications in optical devices, electronics, biotechnologies and catalysis [Alivisatos et al.,1996) Conventional synthetic methods of gold nanoparticles have involved a number of chemical methods [Asmathunisha et al., 2012; Tolles ,1996; Selvakannan et al., 2002; Okitsu et al.,2001]. There is an increasing pressure to develop clean, non-toxic and environmentally benign synthetic technologies. Microbial resistance against heavy metal ions has been exploited for biological metal recovery via reduction of the metal ions or formation of metal sulfides [Stephen and Maenaughton, 1999]. So the attractive procedure is using microorganisms such as bacteria and fungi to synthesize gold nanoparticles recently. In an earlier study, Bacillus subtilis are reportedly reducing Au3+ ions to gold nanoparticles with a size range of 5–25 nm inside the cell walls [Beveridge et al.,1980]. Shewanella algae reduce Au3+ ions forming 10–20 nm gold nanoparticles extracellularly with the assistance of hydrogen gas [Konish et al., 2004]. Fungi such as Verticillium sp.)[Mukherjee et a.,2001] and Fusarium oxysporum [Mukherjee et a.,2002]) and actinomycetes such as Thermomonospora sp. [Ahmadet al.,2003] and Rhodococcus sp. [Ahmad et al., 2003] are also known to synthesize nanoparticles intra- or extracellularly. Many Microorganisms are reportedly reducing silver ions to silver nanoparticles. There are only few reports on biosynthesis of gold nanoparticles using photosynthetic bacteria especially of anaerobic marine environment. In this study, phototrophic bacterium Rhodopseudomonas sp., a typical purple non-sulfur bacterium was used to synthesize gold nanoparticles at room temperature with a single step process.

MATERIALS AND METHODS

Location

The in vitro experiments were conducted out in the microbiology laboratory, Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University.

Chemicals:

All the chemicals used in the experiments were of analytical reagents (AR) grade and distilled water was used throughout the study.

Strain Isolation

The sediment samples were collected from the mudflats of Pichavaram mangrove forest, situated in the southeast coast of India. The samples were brought to the laboratory within 3 hour in a pre-sterilized Mac Cartney bottle, to minimize any change in the micro flora, as suggested by Krishnamurthy et al., (1986). In the present study, the selective enrichment medium used for the purple non sulphur bacteria was obtained through the modification of Pfenning medium. Yeast extract was added as a source of vitamins as described by Pfenning (1978). The medium

contained per liter of aged (approximately 3 months) seawater (30% salinity of the seawater) the following :KH2PO4,1g; NH4Cl,1.5g; MgCl2,0.2g; CaCl2 ,0.2g; Yeast extract,1g; Ascorbic acid ,0.5g; Malic acid ,1g; (Organic carbon compound) NaHCO3,1g. The pH range was between 6.5 and 7. The pinches of mud samples were inoculated into the 36 ml screw cap test tubes which continued the above said enrichment medium. The tubes were then placed at about 25 cm away from the continuous as incandescent light source. As a rule, the development of mixed communities of purple non sulphur bacteria took 3-4 days. In the enrichment cultures, the colour of the cultures varied between brownish red and red. A portion of the above enrichment cultures was then transferred to agar dilution sources for isolation of single strain as suggested by Pfenning and Truper (1981).

Biosynthesis of gold nanoparticles

About 10 grams of wet biomass of Rhodop seudomonas sp. strain was transferred to 50 ml of 1mM chloroauric acid aqueous solution and the whole mixture was kept in a shaker at 280C at 120 rpm. During the process of biosynthesis, all the tubes were observed for visual colour change from yellow to pinkish purple. Then the whole mixture was centrifuged at 5000 rpm for 30 min. One ml of sample was withdrawn and the optical density was taken at a broad range of wavelengths from 300 to 800 nm and a narrow range from 400 to 500 nm using UV–visible spectrophotometer (Elico, Chennai) and plotted the values on a graph.

Characterization of nanoparticle
SEM and EDS Analysis

Scanning Electron Microscopic (SEM) and Energy dispersive spectrum analysis of gold nanoparticles synthesized by Purple non sulphur bacterium Rhodopseudomonas sp. was carried out at Department of Physics, Annamalai university. The specimens were kept on copper grid stained with uranyl acetate and lead citrate and observed under JEOL JEM 100SX Scanning Electron Microscope at 80 Kv.

Dynamic Light Scattering Analysis (DLS)

Dynamic light-scattering measurements were performed for analyzing size groups of nanoparticles using a Nano ZS apparatus at 25 ºC and started 2 min after the cuvette was placed in the DLS apparatus to allow the temperature to equilibrate. Measurements were carried out 24 h after the preparation of the suspensions.

Absorbance spectra of nanoparticles

The optical density was taken at different wavelengths and plotted (Fig. 4.). The absorbance spectra revealed peak at 600 nm for gold nanoparticles for Rhodopseudomonas sp.

FTIR analysis

FTIR analysis, 100 ml of nanoparticle solution was centrifuged at 5000 rpm for 10 min. The supernatant was again centrifuged at 10000 rpm for 60 min and the pellet was obtained. This was followed by redispersion of the pellet of Au-Nps into 1 ml of deionized water. Thereafter, the purified suspension was freeze-dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR.

RESULTS

Maintenance of cultures:

In the present study, the stock cultures were incubated at a moderate light intensity (not close to the bulb, but 50 cm away from the bulb source) as recommended by Biebl and Pfenning (1981). (Fig.1)

Fig.1.Rhodopseudomonas sp. under moderate light intensity

Synthesis of gold nanoparticles

The colour change was noted by visual observation in Rhodopseudomonas sp. when their filtrates were incubated with chloroauric acid solution. The solution showed gradual change from yellowish to pinkish, with intensity increasing during the period of incubation up to12 days Absorbance spectra of nanoparticles

The optical density was taken at different wavelengths and plotted (Fig.2). The absorbance spectra revealed peak at 600 nm for gold nanoparticles for Rhodopseudomonas sp.

Characterization of gold nanoparticles

The shape and size of gold nanoparticles synthesized by Rhodopseudomonas sp. were confirmed by SEM and DLS. The SEM showed the nanoparticles with variable shape, but most of them were cubical in nature. The particle size of gold nanoparticle ranged from 5 to 90 nm. Similarly the DLS also revealed the particle size in the range of 5-100 nm for gold nanoparticles. (Fig.3)

FTIR analysis

The FTIR spectrum revealed the prominent peaks at 3354.21, 2945.30, 2831.50, 1454.33, 1026.06, 756.62 cm-1. These peaks are mostly corresponding to, alcohol (phenolic stretching), carbonyl, amine, and amide. The possible chemical for the reduction of chloroaurate ionmay be

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associate with aromatic and amino groups.(Fig.4)

DISCUSSION

A few papers reported the enrichment procedures for photosynthetic purple non sulphur bacteria(Van Niel 1971; Imhoff,1992). The bacterium was isolated by an agar-shake-dilution purification process following enrichment cultivation as suggested for purple non sulphur bacteria (Rhodopseudomonas sp.) (Pfennig, 1978). The agar shake-dilution technique is a more convenient method than the usually used Hungate rolling tube technique (Pfennig, 1978). In this method, the mixture of melted paraffin wax and paraffin oil (1:3, v/v) is poured onto the surface of an agar tube culture to insulate the medium from oxygen. Under these anaerobic conditions, the anaerobic bacteria grew well and formed colonies (Fig 2). The gold chloride solutions were reduced during exposure to the bacterium Rhodopseudomonas sp.. The colour of the reaction solution turns from pale yellow to ruby red (as shown in Fig. 3.), which indicates the formation of gold nanoparticles extracellularly. The reaction was completed after 24 h of incubation and it indicated a rapid process of nanoparticle synthesis, as comparing to previous works (Shiying He et al.,2007;). The colour of the reaction solution remained ruby red without any changes, and the gold nanoparticles analyzed by UV–Vis spectra and SEM and EDS were stable after 48 h of reaction. Control experiments without biomass addition stayed pale yellow, indicating that the production of gold nanoparticles was obtained by the reduction of microorganisms. Fig. 4 shows the UV–Vis absorption spectra recorded from the gold nanoparticles solution after 48 h of reaction (curve 1). The results indicate that the reaction solution has an absorption maximum at about 550 nm, which can be attributed to the surface plasmon resonance band (SPR) of the gold nanoparticles. The results as shown in Fig. 3 indicate that the solution was stable for at one month with only no aggregation of particles in the solution.

Scanning electron microscopy (SEM) measure ments show the exact shapes of gold nanoparticles. It is clearly seen that nanoparticles are predominantly cubical in nature .As shown in Fig.5.A; well-separated gold nanoparticles with monodispersed are mainly cubical in the size range from 10–20 nm. The Energy dispersive spectrum revealed the presence of gold. The size range of gold nanoparticles (10-20 nm) was confirmed using dynamic light scattering pattern. The FTIR spectrum (Fig.6.) revealed the presence of possible chemicals present along with the gold nanoparticles. The main groups of the enzyme secreted by biomass that may play an important role in reducing the AuCl4 – ions include amino, sulfhydryl and carboxylic groups [Mukherjee et al.,2001; Ahmad et al., 2003;]. And the AuCl4 - ions could bind to biomass through these functional groups. These chemical groups present in the biomass weakens the reducing power of the biomass and allows the gold chloride ions to get closer to the binding sites. So the reaction rate of gold ions is very slow and Au-biomass biosorbent is strong, which would contribute to the formation of cubical morphologies. Previous studies [Mukherjee et al.,2003, Ahmad et al., 2002, Senapati et al., 2005] have indicated that NADH- and NADH-dependent enzymes are important factors in the biosynthesis of metal nanoparticles. Bacterium Rhodopseudomonas sp. is known to secrete cofactor NADH- and NADH dependent enzymes that may be responsible for the bio reduction of Au (3+) to Au (0) and the subsequent formation of gold nanoparticles. The reduction seems to be initiated by electron transfer from NADH by NADH-dependent reductase as electron carrier. Then the gold ions obtained electrons and are reduced to Au (0). The exact mechanism of the reduction of gold ions by the bacterium is yet to be elucidated.

CONCLUSION

The present study showed that the anaerobic bacterium Rhodopseudomonas sp. is able to synthesize gold nanoparticles extracellularly and is quite stable in solution. This is an proficient, environmentally benign and easy method. To best of our knowledge, this is the first work in mangrove derived anaerobe, Rhodopseudomonas sp. in gold nanoparticle synthesis.

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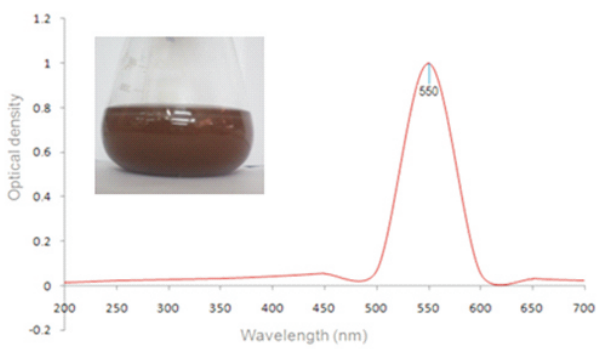

Fig.2. UV-Vis spectrum of plasmon resonance of gold nanoparticles synthesized by Rhodopseudomonas sp..

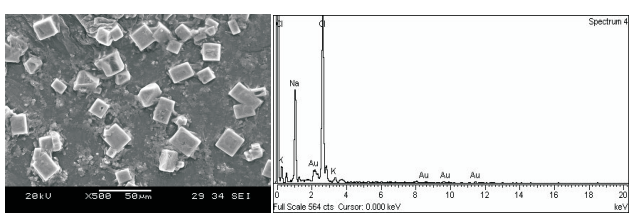

Fig.3. SEM micrograph of gold nanoparticles synthesized by Rhodopseudomonas sp

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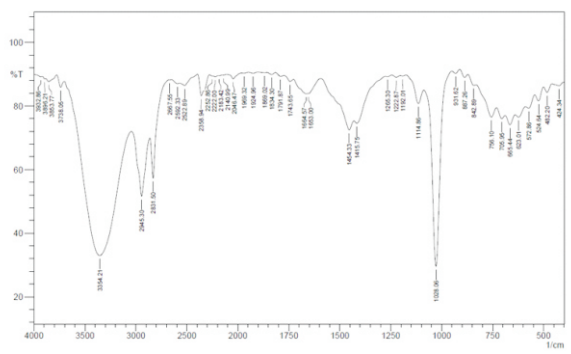

Fig.4. FTIR spectra of gold nanoparticles synthesized by Rhodopseudomonas sp.

REFERENCES

1.A.P. Alivisatos, J. Phys. Chem. 100 (1996) 13226.
2.R. Jin, Y. Cao, C.A. Mirkin, K.L. Kelly, G.C. Schatz, J.G. Zheng, Science 294 (2001) 1901.
3.J. Aizpurua, P. Hanarp, D.S. Sutherland, M. Kall, G.W. Bryant, F.J.G. de Abajo, Phys. Rev. Lett. 90 (2003) 057401-1.
4.K.Kathiresan, N.Sithranga boopathy, Sunil kumar sahu , N. Asmathunisha, K. Saravanankumar, M .Syed Musthafa,. C. Ravinder Singh , S.Kavitha, S.Anandhan, , G.Abirami , K. Kayalvizhi, and Shanmuga arasu,v Bioprospecting potential of mangrove resources ,international journal of current molecular research(2013)1:005-011
5.N .Asmathunisha, K.Kathiresan ,A review on biosynthesis of nanoparticles by marine organisms. Colloids Surf B Biointerfaces. (2013);103:283–287.
6.C.N.R. Rao, A.K. Cheetham, J. Mater. Chem. 11 (2001) 2887. M. Moreno-Manas, R. Pleixats, Acc. Chem. Res. 36 (2003) 638.
7.M. Han, X. Gao, J.Z. Su, S. Nie, Nat. Biotechnol. 19 (2001) 631.
8.W.M. Tolles, Nanotechnology 7 (1996) 59.
9.P.R. Selvakannan, S. Mandal, R. Pasricha, S.D. Adyanthaya, M. Sastry, Chem. Commun. 13 (2002) 1334.
10.K. Okitsu, A. Yue, S. Tanabe, H. Matsumoto, Y. Yobiko, Langmuir 17-(2001) 7717.
11.J.R. Stephen, S.J. Maenaughton, Curr. Opin. Biotechnol. 10 (1999) 230.
12.T.J. Beveridge, R.G.E. Murray, J. Bacteriol. 141 (1980) 876.
13.Y. Konish, N. Deshmukh, T. Tsukiyama, N. Saitoh, Trans. Mater. Res. Soc. Jpn. 29 (2004) 2341.
14.P. Mukherjee, A. Ahmad, M. Mandal, S. Senapati, S.R. Sainkar, M.I.
15.Khan, R. Ramani, R. Parischa, P.V. Ajayakumar, Angew. Chem., Int. Ed. 40 (2001) 3585.
16.P. Mukherjee S. Senapati, A. Ahmad, M.I. Khan, M. Sastry, ChemBiochem 3 (2002) 293.
17.Ahmad, S. Senapati, M.I. Khan, R. Kumar, M. Sastry, Langmuir 19 (2003) 3550.
18.A.Ahmad, S. Senapati, M.I. Khan, R. Ramani, V. Srinivas, M. Sastry, Nanotechnology 14 (2003) 824.
19.C.van Niel, Techniques for the enrichment, isolation, and maintenance of the photosynthetic bacteria. Methods Enzymol (1971). 23: 3–28.
20.Imhoff, J. F. & Tru¨ per, H. G. The genus Rhodospirillum and related genera. In The Prokaryotes,. Edited by A. Balows, H. G. Truper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer. (1992). pp. 2141–2152
21.Pfennig, N.General physiology and ecology of photosynthetic bacteria. In The Photosynthetic Bacteria, (1978) 3–18.
22.R. K. Clayton & W. R. Sistrom. New York: Plenum. Shiying He, Zhirui Guo, Yu Zhang, Song Zhang, Jing Wang, Ning GuBiosynthesis of gold nanoparticles using the bacteria Rhodopseudomonas capsulata Materials Letters61 (2007) 3984–3987
23.V. Armendariz, I. Herrwra, J.R.P. Visea, M.J. Yacaman, J. Nanopart. Res. 6 (2004) 377.
24.A.Ahmad, P. Mukherjee, M. Mandal, S. Senapati, M.I. Khan, R. Kumar, M. Sastry, J. Am. Chem. Soc. 124 (2002) 12108.
25.S. Senapati, A. Ahmad, I.M.I. Khan,M. Sastry, R. Kumar, Small 1 (2005) 517.