Microbial Synthesis of Gold Nanoparticles by Biosurfactant producing 
*Pseudomonas aeruginosa*

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Abstract

There is a need to develop an environmentally friendly method for synthesizing gold nanoparticles using biological sources as potential alternatives to numerous chemical and physical methods which poses harm to the nature. However a significant challenge remains in developing green stabilization techniques that would replace the use of external capping agents. Hence the role of biosurfactants acting as dispersant for gold nanoparticles will be of great importance towards this direction. The objective of the present research is to synthesize stable gold nano-particles with minimal aggregation by biosurfactant producing *Pseudomonas aeruginosa*. This work was focused on the production of stable Gold nanoparticles of uniform size by enhancing biosurfactant secretion by *Pseudomonas aeruginosa* MTCC 424. Secretion of biosurfactant was enhanced by supplementing nutrient broth with additional carbon and nitrogen sources. The synthesis of gold nano-particles was identified by the development of its characteristic colour after being reduced to nano range which was further confirmed by UV Vis spectroscopy. Size and stability of the synthesized gold nanoparticles were analyzed by light scattering studies and zeta potential measurement respectively. It was observed that gold nanoparticles were synthesized by *Pseudomonas aeruginosa* after 24 hours of incubation of biomass (obtained from nutrient broth) with Hydrogen Tetra-chloroaurate. The results demonstrated that gold nanoparticles of smaller size of 12-150 nm with higher stability were synthesized when the nutrient broth was supplemented with an additional carbon source. Higher Zeta potential value of -24 mV was also obtained in it. It was also observed that the same media secreted higher biosurfactant indicating that biosurfactant played an important role in preventing self aggregation. Hence it can be inferred that biosurfactant secreted from *Pseudomonas aeruginosa* act as an excellent capping agent for nanoparticles synthesized by the same microbe. Extensive research must be carried out on the synthesis of nano-particles from biosurfactant producing microbes so that it eliminates the need for extraction of biosurfactants and then adding it as capping agents for nanoparticles stabilization.

**Keywords**: Biosynthesis, nanoparticles stabilization, capping agents, self aggregation and zeta potential

Introduction

Nano-sized materials, known as nanoparticles represents collection of atoms or molecules in the size range of 10^-9 meters. These particles lie within 1 - 100 nm. Nanoparticles are of great scientific interest as they link the gap between bulk materials and atomic or molecular structures. Particularly, Gold nanoparticles (GNPs) are of interest, due to their stability under atmospheric conditions, resistance to oxidation and biocompatibility (Vankatesan et al., 2011). These unique properties can be exploited in a wide range of industrial applications using their optical and electronic properties in optics, electronics, medical diagnostics and treatments, sensors and coatings (Corti and Holliday, 2004).

In view of the current drive to develop eco-friendly processes in material synthesis, the approach of
biological synthesis of nanoparticles is of considerable importance (Tomar et al., 2015). The direction of synthesis has shifted from physical and chemical processes towards ‘green’ chemistry (Vighneshwaran et al., 2007). Consequently, researchers in the field of nanoparticle synthesis have been looking at biological systems (Merroun et al., 2007) such as those that allow a commercially viable and environmentally clean preparation of highly stabilized gold particles (Mukherjee et al., 2008).

In order to control the particle size and shape of nanoparticles various reductants, stabilizers, synthetic surfactants and solvents, etc. have been utilized in nanoparticles preparation. To prevent the agglomeration of metallic nanoparticles, a stabilizing agent such as sodium dodecyl benzyl sulphate (Chen et al., 2003) or polyvinyl pyrrolidone (Frens, 1973) is also added to the reaction mixture, generally the chemical methods are low–cost for high volume; however, their drawbacks include contamination from precursor chemicals, use of toxic solvents, and generation of hazardous by–products which becomes hard to apply medically (Venkatesan et al., 2011). Many microorganisms like bacteria, fungi, yeasts, and algae are good sources of biosurfactants and have several advantages over their chemical counterparts; they are less toxic and biodegradable (Nitschke et al., 2005). Synthetic surfactants being used in nanoparticles synthesis are neither environmental friendly nor cost effective. Therefore, biosurfactants are emerging as a green alternative for nanoparticles stabilization. Biosurfactant mediated nanoparticles synthesis is a recent emergence in the field of nanotechnology (Kasture et al., 2008) and is considered as “green” stabilizer of nanoparticles (Kiran et al., 2010). The biosurfactant-mediated synthesis is better than bacterial-or fungal-mediated synthesis, since biosurfactants reduce the formation of aggregates due to the electrostatic force of attraction and facilitate the uniform morphology of nanoparticles. But biosurfactant mediated synthesis is not economically viable as in this method, commercial biosurfactants are added as stabilizers, so herein, we are trying to establish a cost-effective protocol by synthesizing GNP within the biosurfactant producing organism itself as it will eliminate the addition of any external stabilizers and reducing any side effect on the environment (Banerjee et al., 2015).

In the present work, we have succeeded in synthesizing stable GNPs Pseudomonas aeruginosa MTCC 424 in the intracellular environment. This stability is due to the biosurfactant secreted by the bacterium by altering various media components. The synthesized GNPs were characterized by UV-Visible Spectrophotometer and light Scattering Studies. From the characterization studies we can conclude that GNPs obtained were of uniform size when the media components were altered for enhanced biosurfactant secretion by the organism.

Materials and Methods

Media Components

Glucose, Beef extract, Peptone for bacteriology, Glycine, NaCl, Agar and Hydrogen Tetrachloroaurate (HAuCl₄) AR grade were purchased from Himedia Ltd, Mumbai, India.

Microorganism and Culture Condition

Pseudomonas aeruginosa MTCC 424 strain was obtained from Microbial type collection culture (IMTECH), Chandigarh, India. The strain was cultured and maintained on nutrient agar slants and stored at 4°C. Stock cultures were maintained by subculturing at monthly intervals. The bacteria were inoculated on a nutrient broth at pH 7 and 37°C for overnight.

Production of Biomass

Biosurfactant producing bacteria Pseudomonas aeruginosa was inoculated in 50 ml of nutrient broth
containing Biopeptone (5g/l), Beef extract (3g/l) and NaCl (5g/l) at pH 7. The cultures were incubated with a rotary incubator at 140 rpm, 37°C for 24 hr. After 24 hr of incubation, the cultures were centrifuged at 5000 rpm for 20 minutes at 10°C in order to harvest the biomass. The pellet was collected and washed two times with 0.05 M Phosphate buffer (pH 7) in order to remove any traces of media components that would interfere with the synthesis method.

**Biosynthesis of Gold Nanoparticles**

0.25 gm (wet weight) of biomass was re-suspended in 25 ml of distilled water, pH of cell suspension was maintained at 7 and then required volume of the aqueous AuCl₄⁻ solution was added to maintain final concentration of 1mM and incubated for 24 hr at 150 rpm, 37°C. Control flask (without HAuCl₄) was also kept along with the experimental setup.

**Effect of Biosurfactant Production on Nanoparticle Synthesis**

Biosurfactants reduce the formation of aggregates due to the electrostatic force of attraction and facilitate the uniform morphology of nanoparticles (Kimura et al., 2002). So if we could enhance the production of biosurfactants by the organism we would be able to synthesize GNP’s of uniform morphology with minimal aggregation.

Tugrul and Cansunar (2005) used basal media for biosurfactant production from *Pseudomonas aeruginosa*. Panesar et. al. (2009) investigated that *Pseudomonas aeruginosa* MTCC 2297 was able to grow in a medium containing glycerol and glucose with maximum emulsification activity. Among the various nitrogen sources added glycine and alanine gave similar results, but considering the cost factor glycine was the most effective. Based on the literature survey, Media A, B and C were first checked for GNP synthesis. The compositions of these media are as follows:

<table>
<thead>
<tr>
<th>Media A (g/l)</th>
<th>Media B (g/l)</th>
<th>Media C (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose : 20.0</td>
<td>Glucose: 20.0</td>
<td>Glucose: 20.0</td>
</tr>
<tr>
<td>KH₂PO₄ : 0.7</td>
<td>Peptone : 5.0</td>
<td>Glycine : 2.0</td>
</tr>
<tr>
<td>Na₂HPO₄ : 2.0</td>
<td>Beef Extract: 3.0</td>
<td>Beef Extract: 3.0</td>
</tr>
<tr>
<td>MgSO₄.7H₂O : 0.4</td>
<td>NaCl: 5.0</td>
<td>NaCl: 5.0</td>
</tr>
<tr>
<td>CaCl₂.2H₂O : 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FeSO₄.7H₂O : 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Qualitative Analysis of Biosurfactant Secretion**

For biosurfactant production, *Pseudomonas aeruginosa* was inoculated in all the media which were capable of synthesizing Gold nanoparticles at 37°C, pH 7 and incubated in a rotary incubator at 150 rpm. Among these media, a suitable one was selected based on its ability to secrete maximum amount of biosurfactant by the organism. Biosurfactant activity was detected qualitatively by drop collapse test and oil displacement test.

**Characterization of GNPs**

Characterization is done to establish an understanding and control of nanoparticle synthesis and applications. It is performed by using a variety of different techniques such as UV-Visible spectroscopy, electron microscopy (SEM) and dynamic light scattering (DLS). The cells were ultrasonically disrupted to release the intracellular nanoparticles and separated from other unbound proteins and cell debris and the clear suspension was then used for characterization. The excitation spectra of the samples against blank were measured by UV-Visible spectrophotometer (Lambda 25, Perkin Elmer). The size distribution of nanoparticles were then determined using light scattering studies. The hydrodynamic diameters of the Gold Nanoparticles under investigation were measured using a Malvern Inst.; UK; Nano ZS. Zeta Potential was measured using ZETA SIZER (Nano-ZS), Malvern Instruments, U.K) in order to check the dispersion stability of synthesized gold nanoparticles. This parameter determines the electrophoretic mobility of
the particles within an external electric field, as well as the electrostatic repulsion between particles (or between a particle and a bounding surface) that acts to inhibit or stimulate particle attraction and adhesion (George et al., 2007). The morphological features of the particles were studied with a scanning electron microscope. Jeol JSM-6390 LV SEM was used for visualizing size and shape of synthesized GNPs. Samples were fabricated by dropping the aqueous suspension onto clean electric plate and allowing water to completely evaporate. EDX (Energy disperse X-ray microanalysis) was performed to examine the elemental composition of the sample.

Results and Discussion

Visual observation

The aqueous chloroaurate ions were reduced to metallic gold upon exposure to the bacterial biomass (obtained by inoculating Pseudomonas aeruginosa (MTCC 424) in nutrient broth for 24 hours) as the colour of the reaction mixture turned from pale yellow to dark pink (Fig. -1) after incubation of 24 hours.

Effect of Media on GNP synthesis

The organism was inoculated in Media A as it was reported to secrete maximum biosurfactant, but when this media was assessed for biosynthesis, it gave negative results (no colour change even after 72 hours), hence two other different media (Media B and Media C) which were just the supplementation of additional carbon and nitrogen source to nutrient broth were used for synthesis and light pink colour (Fig.-2a) after 46 hours whereas blue-black (Fig.-2b) colour after 24 hours itself was observed respectively.

The mutual oscillation of electrons of nanoparticle upon interaction with light of suitable energy causes the nanoparticles to attain colour specific to a particular metal (Bhattacharya and Srivastava, 2003). Hence, here in the synthesis of gold nanoparticles was validated by visually monitoring three experimental flasks containing Biomass (from Media A, Media B and Media C) with an auric chloride solution along with their respective control. No change was observed in all the control flasks and experimental flask with Media A, whereas in the other experimental flasks, visual colour change was observed. Such a colour transition indicated the change in the metal oxidation and formation of gold nanoparticles (He et al., 2008). Based on the size, GNPs can turn red, blue, yellow, and other colors. Different thicknesses of materials reflect and absorb light at varying wavelengths (Venkatesan et al., 2011).

Characterization

UV-Visible Spectroscopy

UV–Visible absorption spectroscopy is one of the main techniques to examine the size and shape of the NPs in aqueous suspensions. The UV-Visible spectra of the aqueous reaction mixture were recorded (Fig. - 5). No peak was observed with Media A indicating the absence of GNPs. A strong surface plasmon resonance (SPR) was centered at 543 nm with nutrient broth, 541 nm with Media B and 555 nm with Media C. This resonance range (520 - 550 nm) is a characteristic property of colloidal gold (Mukherjee et al., 2001).

It is well known that the position and intensity of the SPR band depend largely on the size and aspect ratio of gold nanocrystals (Chen et al., 2007). Larger particles and aggregates of nanoparticles show absorption that is more red-shifted as compared to the smaller ones (Bhattacharya and Srivastava, 2003). Since, there is blue shift in the peak (Fig.-5 c) we can conclude that uniform particles of smaller size with minimal aggregation were synthesized with Media B as compared to nutrient broth and Media C. This inference was further supported by light scattering studies. The possible reason for this band shift might be
due to size variation of GNPs caused by the increased secretion of biosurfactant by the bacterium when glucose was used as carbon source and peptone as nitrogen source in Media B, whereas in Media C, due to the presence of glycine being a strong nitrogen source might lead to over expression of enzymes responsible for nanoparticle thus leading to larger particle synthesis with intense aggregation in a very short time. We could infer that there was minimal aggregation with Media B because there is no band broadening (Fig.-5 C) instead a narrow peak was observed.

**Dynamic Light Scattering**

The distribution of the particle showed two ranges of particles located between 2 - 5 nm and 12 to 140 nm.

**Table 1. Values of GNPs synthesized in different media with pH 7 at 37°C**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Media</th>
<th>Zeta Value (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nutrient broth</td>
<td>-23</td>
</tr>
<tr>
<td>2.</td>
<td>Media B</td>
<td>-24</td>
</tr>
<tr>
<td>3.</td>
<td>Media C</td>
<td>-22</td>
</tr>
</tbody>
</table>
with Nutrient Broth (Fig.-6a), whereas particles of 12 - 150 nm with Media B (Fig.-6b) and again a broader size range was observed from 5-400 nm with Media C (Fig.-6c). Thus, it could be inferred that components of Media B played a major role for synthesis of stable GNPs.

**Zeta potential**

The higher zeta-potential value is a key parameter to maintain the stability of suspension because it will make a repulsive force and keep the gold nanoparticles away from each other, which results in a high stability of suspension. Higher Zeta potential value of -24 mV was obtained in Media B (Table - 1).

**SEM and EDX Analysis**

The white spots in the image indicate gold nanoparticles, which were further confirmed by SEM and EDX analysis of that spectrum (Fig.- 3).

**Detection of Biosurfactant Secretion**

Based on the absorbance values, Media B was found to be the most suitable one as it gave the maximum surface plasmon peak at a smaller wavelength in comparison with other media, thus indicating synthesis of smaller sized particles which was further confirmed by light scattering studies.

Table - 2. Observation of Drop Collapse Test after 24 hours of incubation

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Media Supernatant</th>
<th>Collapse time (Seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nutrient Broth</td>
<td>54</td>
</tr>
<tr>
<td>2.</td>
<td>Media B</td>
<td>4</td>
</tr>
<tr>
<td>3.</td>
<td>Media C</td>
<td>5</td>
</tr>
</tbody>
</table>

**Fig.- 5: UV-Visible spectra of GNPs synthesized from (a) Nutrient Broth (b) Media A (c) Media B and (d) Media C**

**Fig.-6. Size Distribution of GNPs synthesized from (a) Nutrient Broth (b) Media B (c) Media C**
Those Media (Nutrient broth, Media B, Media C) which were capable of biosynthesis were checked for biosurfactant production. A clear halo zone was observed when oil displacement test was performed with the culture supernatant of all these media, thus indicating a positive result, whereas when distilled water as a control was used, it remained in beaded form (Fig.- 4).

Then drop collapse test was also performed. The culture supernatant from both Media B and Media C took almost similar time to collapse in comparison with nutrient broth, which took longer time to collapse (Table - 2). It was observed that both Media B and Media C an gave almost similar amount of biosurfactant.

**Conclusion**

We report here a green chemistry approach using *P. aeruginosa* in the synthesis of stable gold nano particles at 37°C without using any external stabilising agents. Media B (Nutrient Broth supplemented with additional Carbon source) was found to be the best as the biomass from this media was able to synthesize GNP of uniform size with minimal aggregation. The higher biosurfactant secretion in Media B could be the reason for minimal aggregation as biosurfactant could act as dispersant. In the present research, biosurfactants enhanced the zeta-potential. Thus, it was inferred that biosurfactant producing organisms could be used as a greener alternate for achieving size controlled synthesis. This approach of synthesizing GNP from biosurfactant producing microorganism is general and cost effective as it eliminates the use of any external capping agents.

**References**


Estimate of Thiolate-modified Gold Nano particles.


