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**R M Mendoza[1], Z K Baybay[2], L M Fernando[2], A D Montecillo[1], L L Ilag[3] and L C Villegas[1]**

[1]Microbiology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Laguna, Philippines
[2]Nano-Biotechnology Innovations and Translational Research Laboratory, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños, Laguna, Philippines
[3]Xerion Limited, Victoria, Australia

E-mail: rmmendoza7@up.edu.ph

**Abstract**. The wide applications of gold nanoparticles in fields such as electronics and biomedicine (targeted-drug delivery) have caught the interest of many researchers. In this study, *Serratia marcescens* NBL1001 was screened for its ability to synthesize gold nanoparticles (AuNP). Colorimetric change from light yellow to purple, 24 h after the supplementation of 1mM tetrachloroaric acid ($HAuCl_4$), indicated production of AuNP. *UV-Vis* scanning spectrophotometry of bacterial suspension, cell-free supernatant and reconstituted cell pellets of *S. marcescens* NBL1001 showed absorbance peaks characteristic of AuNP (500-600 nm) at 554 nm, 556 nm and 550 nm respectively. Gold nanoparticles with sizes ranging from 11.78 to 46. 03 nm with mean size of 25.28 nm (n=30) were observed under the scanning electron microscope and the presence of elemental gold ($Au^0$) was confirmed by energy dispersive X-ray spectroscopy.

## 1. Introduction

Nanotechnology has generated great enthusiasm over the recent years because of its expected impacts on energy, chemical, electronics, space industries, and medicine [1]. Nanotechnology involves the manipulation of nanomaterials which include the nanoparticles that have attracted interest due to their unique optical, thermal, electrical, chemical and physical properties [2-4].

Nanoparticle synthesis has garnered the interest of many researchers because of its wide application in fields such as electronics, phytochemical, biomedicine and chemistry [5]. Gold nanoparticles in particular have applications on medicine such as in targeted-drug delivery and cancer therapy [5]; as potential antimicrobial agents to combat the increasing occurrence of antimicrobial resistance to currently available antibiotics [6]; and on the environment, as biosensors that can detect the presence of heavy metals [7]. However, majority of the manufacturing techniques usually employed for the synthesis of nanoparticles are capital-intensive, inefficient in material and energy use, and has environmental concerns, [2]. Consequently, the need to develop clean, non-toxic and

environmentally benign synthesis procedures emerged. Hence, biological synthesis of nanoparticles came into play [3].

Green chemistry defined as the use of biological systems in the synthesis and assembly of nanomaterials is a relatively clean, non-toxic, and environment-friendly procedure [4]. In contrast, conventional chemical synthesis techniques make use of toxic chemicals which engender serious environmental concerns [1]. Several organisms such as bacteria, fungi, algae, and plants have been used in the biosynthesis of many nanomaterials such as gold, silver, cadmium sulphide silica, among others [8]. Majority of these nanoparticle-producing organisms are microorganisms.

*Serratia marcescens* is a Gram-negative bacillus belonging to the family Enterobacteriaceae. Although it has been associated with nosocomial infection and known to have intrinsic and acquired resistance to antibiotics, it has been a subject to a number of studies due to its antimicrobial and industrial applications. Very recently, this has been found to have application in nanotechnology as it was found to produce silver and gold nanoparticle [9,10]

Biologically-mediated synthesis of gold nanoparticles offers a clean, environment-friendly, and efficient production procedure, since biological systems are easy to handle and grow, and do not require the use of toxic chemicals as reducing agents [4, 11]

This study demonstrated the ability of *S. marcescens* NBL1001 to biomediate the synthesis of AuNP; and characterized the AuNP produced by *S. marcescens* NBL1001 via *UV-Vis* spectrophotometry, Scanning Electron Microscopy, and Energy Dispersive X-ray spectroscopy.

## 2. Materials and methods

### *2.1. Cultural characterisation*

*S. marcescens* NBL1001 was obtained from the culture collection of the Microbiology Division of the Institute of Biological Sciences, University of the Philippines Los Baños. The culture was purified in Luria-Bertani agar (LA) plates. The colony characteristics such margin, elevation, pigment production, and texture were observed. Gram reaction of the putative S. *marcescens* NBL1001 was also verified.

### *2.2. Biochemical characterisation*

Biochemical tests using the Analytical Profile Index (API) 20E kit (API20E Biomerieux, USA), was done following the manufacturer's instruction manual. Oxidase test was done using a strip of sterile filter paper on which a drop of oxidase reagent was mixed with a loopful of *S. marcescens* NBL1001 culture. Catalase test was conducted by mixing hydrogen peroxide ($H_2O_2$) with a loopful of culture and was observed for bubble (gas) formation [12].

### *2.3. Molecular Characterization*

#### *2.3.1. DNA extraction*

Genomic DNA (gDNA) was extracted using the *QIAamp DNA Mini Kit* (Qiagen, USA), following the manufacturer's protocol.

#### *2.3.2. 16s rRNA gene amplification through Polymerase Chain Reaction.*

To verify the identity of *S. marcescens* NBL1001, the *16s rRNA* gene was amplified [13]. The PCR cocktail contained the following: 0.2 µM primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') [13, 14], 2X PCR mix (1st Base Laboratories, Malaysia), and 1 µL (50 ng) of extracted gDNA. Tubes were placed in thermocycler (Veriti™) and allowed to run 35 cycles, with initial denaturation at 95°C for 5 min, denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. PCR products were run in 1% agarose gel with Good view stain (1st Base, Malaysia) using Optima Mupid®-2pus Submarine-type electrophoresis system, with 1-kb KAPA Universal ladder serving as the molecular weight marker. Bands were visualized using *Biorad ChemiDoc™ MP System* (ThermoFisher, USA).

*2.3.3. DNA sequencing and BLASTn analysis*

The amplicon was sent to 1st BASE Laboratory Sdn Bhd (Malaysia) for sequencing. The generated sequence was subjected to BLASTn (https://blast.ncbi.nlm.nih.gov) analysis to verify the identity of *S. marcescens* NBL1001. Sequence was analyzed by *MEGA 7.0 software* [15].

*2.4. Gold nanoparticle (AuNP) Production of S. marcescens NBL1001*

*S. marcescens* NBL1001 was cultured in two 8-dram vials containing 10 ml each of minimal medium (M9) broth (5x M9 salts ($Na_2HPO_4$. $7H_20$, 64 $g.L^{-1}$; $KH_2PO_4$, 15 $g.L^{-1}$; NaCl, 2.5 $g.L^{-1}$; $NH_4Cl$, 5 $g.L^{-1}$; in deionized water); 20% glucose, 1M $MgSO_4$, 2 $ml.L^{-1}$; $CaCl_2$, 100 $ul.L^{-1}$) at ambient room temperature with shaking for 24 h. The cells from one vial were harvested by centrifugation at 5000 x g for 15 min. Supernatant was filtered through a 0.22 µm sterile membrane filter (*Whatman*, Merck, USA) to another sterile dram vial, and the cell pellet was resuspended in 5 ml sterile distilled water. Tetrachloroauric acid ($HAuCl_4$) was added to the bacterial suspension, filtered supernatant, resuspended cell pellet and uninoculated M9 broth to final concentration of 1mM. The vials were then incubated at ambient room temperature with shaking for another 24 h. The solutions were observed for color change from yellow to red/purple and *UV-Vis* spectroscopy using *Genesys 10S UV-VIS Spectrophotometer* (Scanning mode; scan speed: fast; interval: 1.0 nm*), (Thermo Scientific,* USA) at wavelength of 400-700 nm; where the maximum absorbance for gold nanoparticles was expected at ~500 to 600 nm. Sterile M9k broth with 1.0mM $HAuCl_4$ was used as blank.

*2.5. AuNP characterization via scanning electron microscopy and energy dispersive X-ray spectroscopy*

The AuNP produced from the cell-free extract of *S. marcescens* NBL1001 were sent to Advanced Device and Materials Testing Laboratory (ADMATEL) in Bicutan, Taguig Philippines for Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X-ray Spectroscopy (EDX) for characterization. SEM produces electron images which helps determines the morphology (shape) of AuNP produced while EDX maps elements and confirms presence of elemental gold in the sample.

## 3. Results and discussion

*3.1. Confirmation of S. marcescens NBL1001*

The *S. marcescens* NBL1001 from the collection of Microbiology Division of the Institute of Biological Sciences, University of the Philippines Los Baños was confirmed to be Gram-negative with short rod cells. The colonies observed on LA plates were round with entire margin and umbonate elevation, with smooth and shiny texture, and exhibited intense red pigmentation due to prodigiosin.

**Figure 1**. A 24-h culture of *S. marcescens* NBL1001 on LA plate showing colony morphology.

**Table 1.** Biochemical reactions of *S. marcescens* NBL1001

| Biochemical Tests | Results | Biochemical Tests | Results |
|---|---|---|---|
| B-galactosidase | + | Glucose Fermentation | + |
| Arginine Dihydrolase | + | Mannitol Fermentation | + |
| Lysine Decarboxylase | + | Inositol Fermentation | - |
| Ornithine Decarboxylase | + | Sorbitol fermentation | + |
| Citrate Utilization | + | Rhamnose fermentation | - |
| H2S production | - | Saccharose fermentation | + |
| Urease | - | Melibiose fermentation | + |
| Tryptophan Deaminase | + | Amygdalin fermentation | - |
| Indole production | + | Arabinose fermentation | - |
| Acetoin production | + | Oxidase | - |
| Gelatinase | + | Catalase | + |

Biochemical tests using API 20E showed 95.9% similarity with *S. marcescens* in APIweb database, with results (Table 1) similar with the reported biochemical characteristics of *S. marcescens* [16], except on the results on, arginine dihydrolase, urease, indole production, and tryptophan deaminase. Blastn analysis of *16s rRNA* amplicon of the isolate confirmed 100% similarity with *S. marcescens* in database. Although the API classification system is based on colorimetric reactions and thus highly subjective, the data gathered from characterization and classification using the API system can support the data obtained from the molecular characterization of organism.

*3.2. AuNP Production*

*S. marcescens* NBL1001 demonstrated AuNP production. The color change from yellow to purple in the cell-free supernant, reconstituted cell pellets, and bacterial suspension, supplemented with $HAuCl_4$ after 24 h indicated AuNP production (Figure 2).

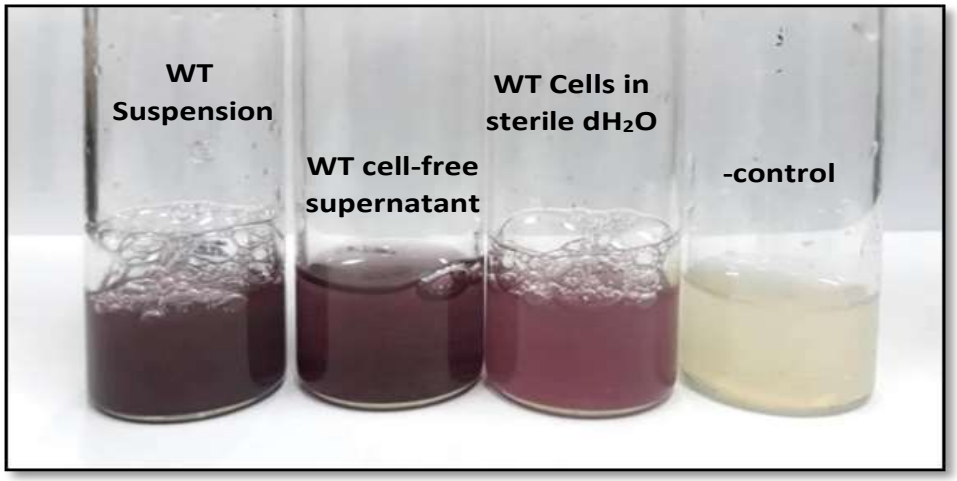


**Figure 2.** Gold nanoparticle production by *S. marcescens* NBL1001 supplemented with 1.0 mM $HAuCl_4$, after 24 h of incubation with shaking at ambient room temperature.

The intra- and extra- cellular production of AuNP by *S. marcescens* has been previously documented to occur over a range of pH, and was observed to be faster in cell-free extract than that in the biomass of *S. marcescens* [10]. In the study [10], AuNP production occurred 24 h after supplementation with 2.5 mM $HAuCl_4$ in the cell-free extract of *S. marcescens* while AuNP production with the biomass of *S. marcescens* took 6 days. However, in the present study, AuNP is produced 24 h after supplementation of 1 mM $HAuCl_4$ solution in both cell-free extract and biomass

of *S. marcescens* NBL1001 wherein the initially light yellow solution of cell-free supernatant with gold, and yellow suspension of cell biomass and suspension with gold, turned violet (Figure 2). Such color change from red to violet complex has generally been considered as an indication of the reduction of $HAuCl_4$ to elemental gold $Au^o$ [10].

The reduction reaction of gold solution is expressed as:

$$HAuCl_4 + 3e^- \rightarrow Au + 4Cl^- + H^+ \quad (1)$$

The characteristic red to violet complex exhibited during the production of AuNP, is due to their photophysical response that is not observed in bulk gold. This can be explained by the property of colloidal gold nanoparticles known as the localized surface plasmon resonance (LSPR) [17]. LSPR is dependent on several factors such as size, shape, structure, metal type, etc., of nanoparticles. For example, in AuNP of smaller size ~10 nm, LSPR causes absorption of light in the blue-green portion of the spectrum while reflection of the red light yields a red complex. The LSRP phenomenon, when upon exposure to different wavelength of lights, collective coherent oscillation of free electrons of the metal particle is induced [11, 17]. The electron oscillation causes a charge separation with respect to the ionic lattice, hence forming a dipole oscillation along the direction of the electric field of the light. The amplitude of the oscillation reaches a maximum at a specific frequency, the surface plasmon resonance, which induces a strong absorption of the incident light hence, can be measured by a *UV-Vis* spectrophotometer [17].

To verify the production of AuNP from the reduction of gold ions, the AuNP produced using the cell-free supernatant, bacterial suspension, and reconstituted cell pellet of *S. marcescens* NBL1001 were subjected to *UV-Vis* spectrophotometry. Results showed that reconstituted cells of *S. marcescens* NBL1001 had the maximum absorption at ~550 nm with absorbance of 2.43, meanwhile, bacterial suspension had the highest peak at ~550 nm with absorbance value of 2.2; and cell-free supernatant at ~560 nm with absorbance of 0.9 (Figure 3). *UV-Vis* spectrophotometry is one of the most important techniques in identifying the formation and stability of gold nanoparticles produced in aqueous solution [10]. AuNP are known to exhibit a maximum absorption in the range of 500-600 nm, and this was observed in the AuNP produced by the bacterial suspension, cell-free supernatant, and reconstituted cell biomass of *S. marcescens* NBL1001 (Figure 3).

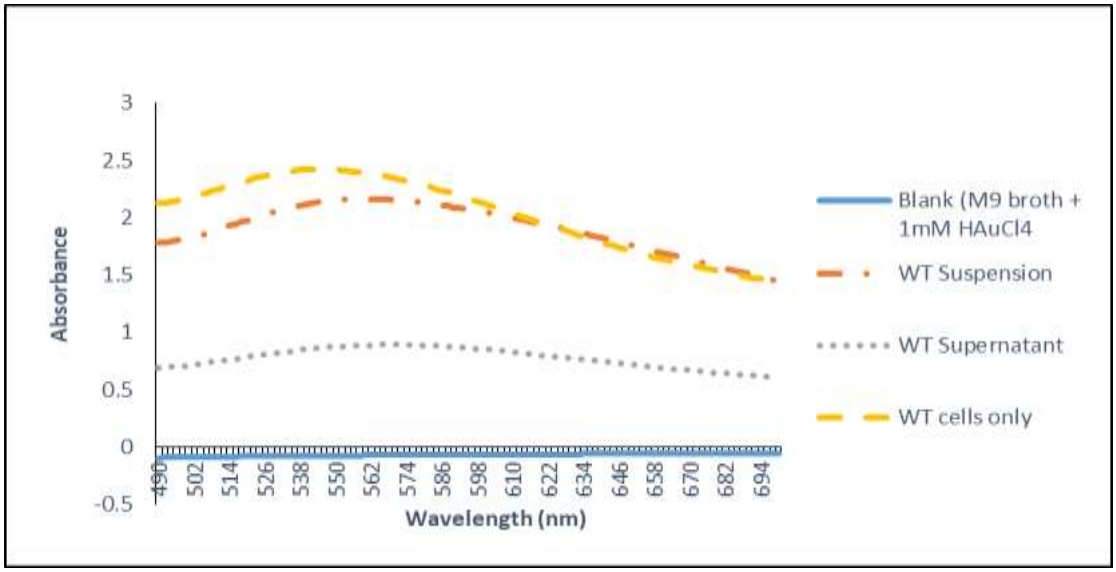


**Figure 3.** Absorbance spectra (400-700 nm) of bacterial suspension, filtered supernatant and reconstituted cell biomass of *S. marcescens* NBL1001.

*3.3. Aunp characterization via scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX)*

*3.3.1 Scanning electron microscopy.*

The sizes of the AuNP produced by cell-free extract of *S. marcescens* NBL1001 ranges from 11.78 - 46.03 nm with mean size of 25.28 nm (Figure 4). Nanoparticles within the 40-60 nm size range are suitable for applications in nano-medicine such as in cancer detection and treatment [10]. SEM micrograph shows that some of the AuNP produced by the cell free supernatant of *S. marcescens* NBL1001 were within this range. The AuNP produced by the cell-free extract of *S. marcescens* in literature were predominantly nanosphere of different sizes [10]. It has been shown that the size of AuNP may be altered with changing pH [10]. In the present study, given that the effect of factors such as pH and temperature on AuNP synthesis by our isolate were not yet studied, it is possible that this *S. marcescens* NBL1001 can produce AuNP that have potential uses in cancer therapy.

Smaller AuNP (10-40 nm) have been observed to have highest absorbance at wavelength 515 nm to 540 nm while larger AuNP (50-100 nm) are associated with wavelength that ranges from 550 nm to 570 nm [10]. However, in this study, the sizes of AuNP produced by cell free-supernatant of *S. marcescens* NBL1001 ranged from 10-50 nm (Figure 3) while the maximum absorption of cell-supernatant was recorded at ~560 nm (Figure 2). This may be due to difference between the times the *UV-Vis* and SEM analyses were done. The AuNP may have changed in shape during the time interval which could have led to the difference in maximum absorptions [17]. Another possibility is that, the distribution of bigger AuNP (50-100 nm) may be dominant compared to the smaller ones.

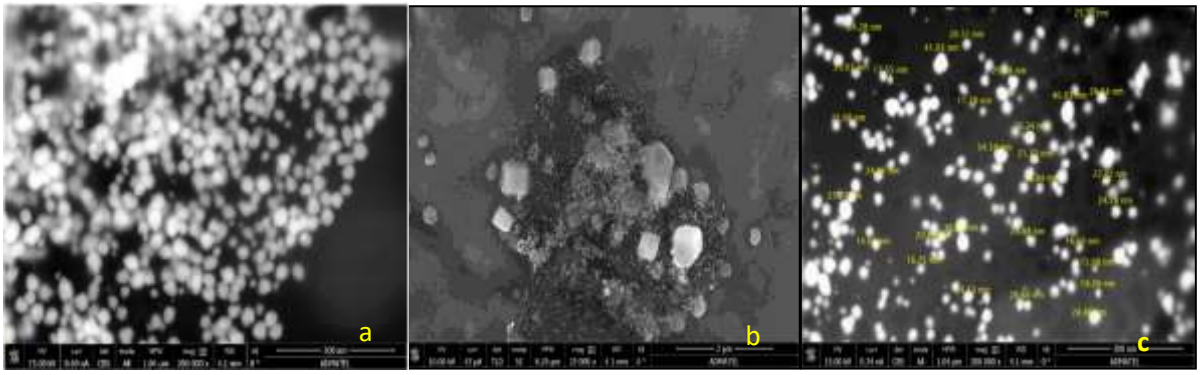


**Figure 4.** SEM micrograph of AuNP produced from cell-free/filtered supernatant of *S. marcescens* NBL1001 with sizes ranging from 11.78- 46.03 nm with mean size of 25.28 nm (n=30) taken at a, c (200, 000x) and b (25, 000x) magnification.

*3.3.2. Energy dispersive X-ray spectroscopy (EDX).*

This verifies the reduction of $HAuCl_4$ to elemental gold with 57.9% (% weight) detected in the sample. The other elements such as carbon, oxygen, sodium, chlorine, and copper, may have been from the matrix used in sample preparation. The copper and carbon detected may be from the sample grid, while chlorine, potassium, phosphorus and sodium elements may have been from the traces of salts present in the culture broth.

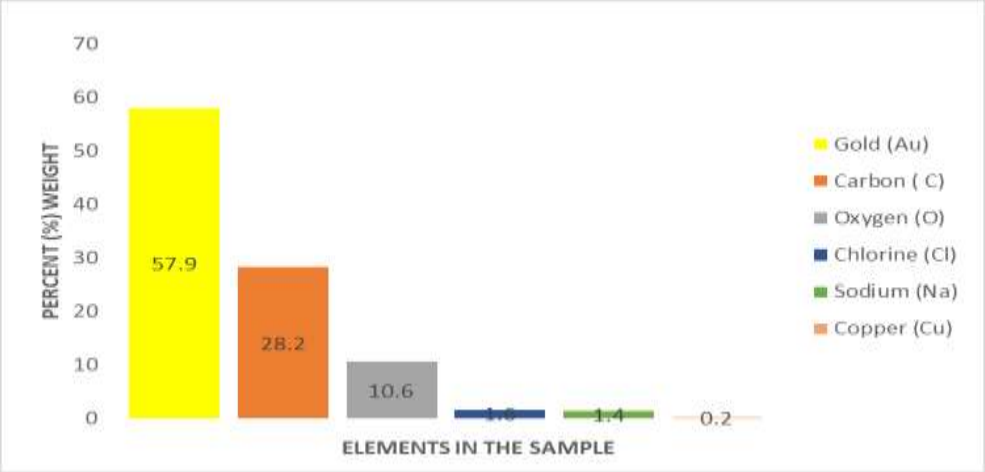


**Figure 5.** Percent (%) weight of elements detected by EDX analyses indicating the presence of elemental gold ($Au^o$) with the highest value of 57.9% in the cell-free spent supernatant of *S. marcescens* NBL1001.

## 4. Conclusion

*S. marcescens* NBL1001 demonstrated its ability to produced AuNP. This ability was supported by the detection and characterization of AuNP from bacterial suspension, cell-free supernatant, and cell pellets, via *UV-Vis* spectrophotometry, SEM, and EDX spectroscopy. These results open possible optimization of biological AuNP synthesis. In addition, genetic manipulation of *S. marcescens* NBL1001 for better AuNP production phenotype may be considered.

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