Mycosynthesis of biocompatible gold nanoparticles using *Penicillium sp* for bromothymol blue degradation

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ABSTRACT

Introduction and Aim: The Biosynthesis of Gold nanoparticles (Au NPs) is an eco-friendly, cost effective and nontoxic alternative to chemical and physical methods. In the present study synthesis of Au NPs was performed by using a fungi *Penicillium sp*. isolated from agriculture soil.

Materials and Methods: Fungi was isolated from the agricultural field and inoculated into Sabouraud Dextrose broth and incubated at 28º C in a shaker at 180 rpm for 96 to 120 hours. After incubation, the fungal culture was filtered and centrifuged, obtained fungal cell free extracts treated with 1mM gold salt ($HAuCl_4$). The synthesis of Au NPs was confirmed by UV–visible spectroscopy and particles size was measured using Dynamic Light Scattering (DLS). Haemolytic assay of Au NPs was carried out using Chicken RBCs and results measured at 540 nm in UV-visible spectrophotometer. To study catalytic activity, Bromothymol blue (BB) was subjected to reduction by using sodium borohydride ($NaBH_4$, 5.28X10-2 M) in the presence of Au NPs. Then the color change was monitored by visual observation.

Results: The synthesis of Au NPs was preliminary observed by a color change from yellow to purple and confirmed by a peak at 560 nm using a UV–visible spectroscopy. The DLS analysis showed that the Au NPs were poly-dispersed and size ranges from 130 to 150 nm. The biosynthesised Au NPs was studied for their biocompatibility and dye degradation properties.

Conclusion: The obtained results revealed that biosynthesized Au NPs shows a minimum level of toxicity to chicken erythrocytes and good catalytic activity towards the degradation of hazardous dye bromothymol blue. These nanoparticles could be potentially useful in various applications in medical and environmental fields.

Keywords: Biosynthesis; *Penicillium sp.;* gold nanoparticles; catalytic activity; haemolysis.

INTRODUCTION

Nanotechnology is an immensely emerging concept in the field of science and technology. Nanotechnology defined as nanoparticle that acts as a whole unit in transport and other properties. The term nanotechnology was entitled by famous lecture, Richard Feyman of the American Institute of Technology. The word 'nano' means extremely dwarf or very small it derived from a Greek word it indicates 10^{-9}or billionth of a meter (1, 2). Physical, chemical and biological methods are traditionally used for the synthesis of metal nanoparticles. In the chemical methods, chemical reagents caused environmental hazards and physical methods main disadvantages are cost effective (3). However, the biological method is a good alternative for the synthesis of different metal nanoparticles with less cost and eco-friendly routes (4). Microorganisms such as bacteria, fungi, yeasts and algae are used to synthesize the Au NPs. Many fungi are a better resource for the synthesis of Au NPs because of their several superiorities, such as efficient synthesis process, high ion concentration

tolerance, high yields and it can synthesize both intra and extracellularly (5). Previous studies have shown that some fungi like *Fusarium acuminatum* (6), *Penicillium citrinum* (7), *Aspergillus fumigatus* and *A. flavus* (8) and *Alternaria alternata* (9) could synthesis nanoparticles.

Au, Te, Pt, Ag, Zn, Cd and many metal nanoparticles, among these metal nanoparticles Au have many biological properties. Au NPs have more advantages in biomedical fields over other metallic nanoparticles due to their non-cytotoxicity and biocompatibility. This markedly leads to a wide range of applications in bio-imaging, bio-labelling, drug delivery, antimicrobial therapy, cancer treatment, biosensors and catalytic applications (10, 11).

Recent days have witnessed a shortage of safe and clean water in India and the world. The water reservoirs are facing several challenges due to the industrialization, urbanization, agriculture and mining activities (12, 13). The disposal of wastewater from dying industries has thus attracted more attention in order to a sustainable water system (14).

In the present work, reported the synthesis of Au NPs by supernatant of *Penicillium sp.* as reducing and stabilizing agent. The active isolate was characterized based on morphological features and microscopic observations. Synthesis of nanoparticles was confirmed by UV-spectrophotometer and size distribution analysis by DLS. Further mychosynthesised Au NPs studied for their haemolytic and catalytic properties.

MATERIALS AND METHODS

Fungal strain and their growth conditions

Soil sample was collected from the agricultural field near Chikka Aluvara, Kodagu, Karnataka and it was serially diluted and known dilution was plated using Sabouraud Dextrose agar and incubated at room temperature for 5 to 7 days. After incubation, the fungal culture was inoculated in 500 ml flask containing 200 mL of sterile SD Broth with final pH 5. These inoculated flasks were incubated at 28º C in a shaker at 180 rpm for 96 to 120 hours.

Extracellular biosynthesis of Au NPs

After incubation, the fungal culture was filtered and centrifuged at 8,000 rpm for 15 min at 4^0 C in a cooling centrifuge. Immediately, obtained fungal cells free extracts were used for Au NPs synthesis. For Au NPs synthesis 2 mL of fresh supernatant (obtained by centrifugation) was added to 3 mL of 1mM gold salt ($HAuCl_4$) in a test tube and two controls were used, one as 1mM $HAuCl_4$ which lacked the fungal cells extract and other control only fungal cells extract. All the experimental samples were incubated at 37 ^{0}C for 48 hours. Synthesized Au NPs were separated by centrifugation at 14,000 rpm for 15 min at 4 ^{0}C. After centrifugation metal nanoparticle were collected from the bottom of the centrifuge tube. Collected nanoparticles were repeatedly redisposed with sterile water followed by alcohol wash to remove biological molecules. The well purified colloidal solution of Au NPs was then heat dried using an oven at 50 ^{0}C for overnight. The dried sample used for further analysis.

Characterization of Au NPs

Bio-reduction of Au NPs primarily monitored by visual observation and qualitative analyses was done by using UV-Vis spectrophotometer ranging between 300 to 800 nm at a resolution of 1 nm. The aqueous colloidal suspension was added into a clean quartz cuvette and spectral measurements were taken immediately. The surface Plasmon resonance (SPR) peaks were assessed for confirmation of Au NPs synthesis. Dynamic Light Scattering (DLS) was used to determine the particles size distribution profile. This is the most common technique used to determine the particles size and dispersity in colloidal suspensions (15).

Hemolysis assay

Hemolysis study was performed according to the previously reported protocol (16). The chicken blood sample was used to carry out the

hemolytic assay of Au NPs. Chicken RBCs were separated by centrifuging at 1500 rpm for 10 min and removed supernatant. The obtained pellet was further washed for several times with sodium saline (0.85%). The pellet was made up to 20 ml volume with saline and 1 ml of the RBCs were mixed with biogenic Au NPs. 1 ml of distilled water with the same volume washed RBC used as positive control and 1 ml of washed RBC with saline used as negative control. All the test and control samples were incubated for 4 h at 37 °C. After incubation of test samples, the mixtures were again centrifuged at 14,000 rpm for 10 min to remove the nanoparticles. The supernatants were measured at 540 nm in UV-visible spectrophotometer.

Catalytic study of Au NPs

Catalytic activity was done by using previously reported protocol (17). Bromothymol blue (BB) was subjected to reduction by using sodium borohydride ($NaBH_4$, $5.28X10^{-2}$ M) in the presence of Au NPs. 5 ml of BB (10^{-5} M) was mixed with 1.5 ml of freshly prepared 10^{-2} M $NaBH_4$ and required quantities of biosynthesized Au NPs was mixed. Then the color changes were monitored by visual observation. Control was incubated at the same experimental conditions without adding nanoparticles.

RESULTS AND DISCUSSION

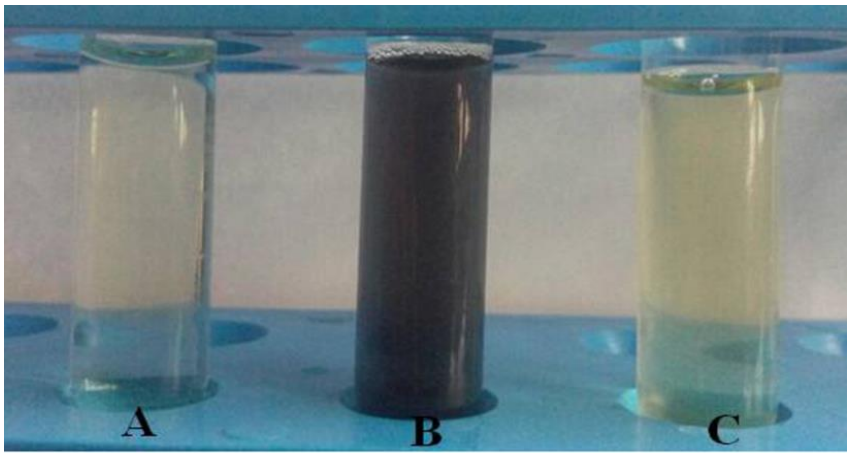

Fig.1: Visual observation of *Penicillium sp.* filtrate exposed to 1 mM gold salt concentration. **A**. Control without fungal supernatant, **B.** With 1 mM gold salt concentration,**C.**Culture supernatant withoutgold salt**.**

Visual observations

The aqueous 1mM $HAuCl_4$ was added to the cell free cultures of *Penicillium* sp. and the color of the reaction mixture changed from light yellow to purple color (Fig 1) which indicated the formation of Au NPs. The color change was monitored visually which signifies the bio-reduction of gold ions and the formation of Au NPs. No color change was observed in control tubes that were incubated at the same experimental conditions without adding fungal filtrate. Similarly, in previous report aqueous $HAuCl_4$ was added to the leaf and twig extract of *R. tuberosa* and *P. acidus* and reaction mixture color turned from light green (plant extract) to purple color which indicated the formation of Au NPs (18).

Table1: *In vitro*Haemolyticactivity of goldnanoparticles and percentagesof haemolysis.

Sample	Content	UV-visblereadingat 540 nm	% of Haemolysis
Negativecontrol	Saline + RBC	0.012	0.6%
Positivecontrol	Distilledwater + RBC	2.107	100%
Test sample	Au NPs + RBC	0.071	3.3%

UV–Visible spectroscopy

Formation of Au NPs was detected by spectral analysis under UV-Vis spectrophotometer. The SPR peak of Au NPs usually has a range of 530–580 nm in colloidal solutions depending on the size and shape of the nanoparticles. With the increase in the nanoparticles size, the SPR peak extends towards longer wavelength. In the present study showed a broad SPR peak in the range of 530–560 nm as shown in the Fig 2, which is indicated for the synthesis of poly-dispersed nanoparticles. According to the previous study, the UV-Vis spectra were recorded after the completion of the reaction, showed a distinct absorption peak at 543 nm which was similar to the present study (19).

Dynamic Light Scattering

DLS analysis was used to measure the size of the particles in colloidal solutions synthesized by different protocols. The particle size distribution versus intensity of the graph is shown in Fig 3. The average particle size of synthesized Au NPs by *Penicillium* sp. was found to be 130 to 150 nm. Generally, the larger particle size observed by DLS is due to the bioorganic compounds enveloping the core of the biogenic Au NPs (20).

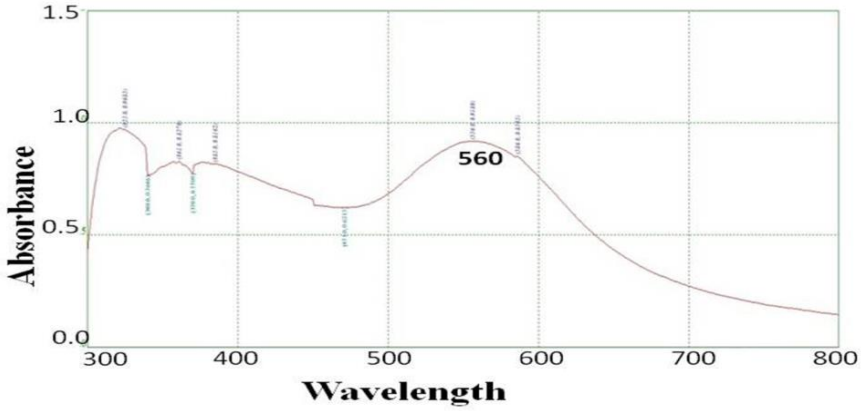

Fig. 2: UV–vis peak recorded after formation of Au NPs by culture supernatant of *Penicillium sp.*

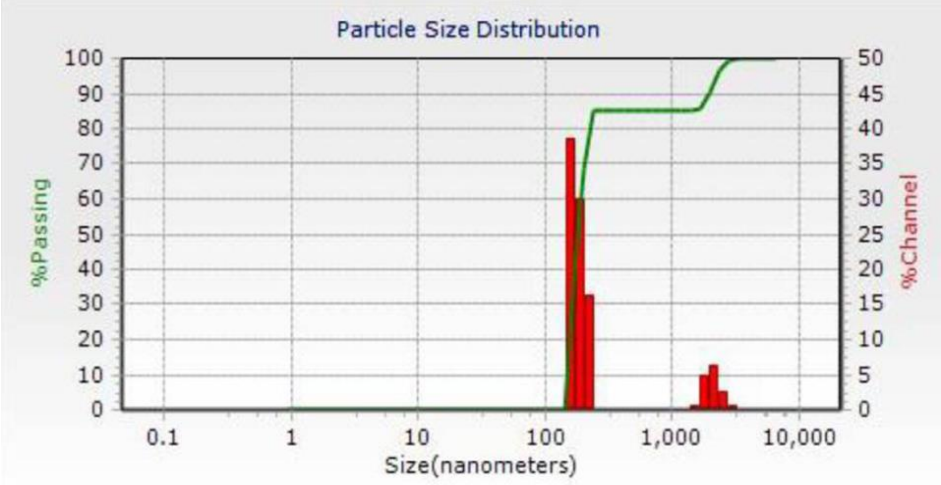

Fig 3: DLS analysis shows size distribution of bio-synthesized Au NPs from *Penicillium sp.*

Properties of Au NPs

Hemolytic property

Toxicity of biogenic Au NPs towards chicken red blood cells was screened using *in-vitro* hemolytic assay. In the present study, Au NPs shows a minimum level of toxicity to erythrocytes (Fig 4). The obtained results show that 3. 3% of hemolysis was observed in the tested samples (Table 1). In the present study the percentage of hemolysis is very low compared to the positive control, so synthesized gold nanoparticles are biocompatible. These biogenic Au NPs could be used for biomedical applications such as drug delivery, gene delivery, cancer therapy etc. According to

Aseichev *et al.,* 2013 Au NPs of small size (5, 10, and 20 nm) showed slightly increased hemolysis in comparison with control during their incubation (21).

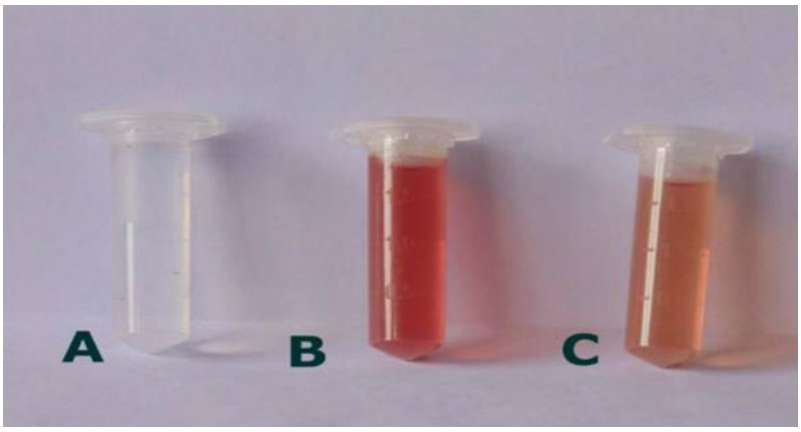

Fig 4: Hemolytic property of Au NPs. (**A-**Negative control, **B**-Positive control, **C**- Test sample).

Catalytic activity

A potential advantage of synthesized Au NPs as a catalyst was determined by the reduction of hazardous environmental pollutant Bromothymol Blue. The catalytic activity of Au NPs was evaluated by using $NaBH_4$ as a reducing agent. After adding this agent, the color of the BB changes from yellow to Blue. When a few drops of Au NPs were added to the aqueous solution of Bromothymol blue and $NaBH_4$ the reduction process found to be accelerated. The color of the aqueous solution turned colorless in a fraction of seconds (Fig 5). This result indicates that Au NPs synthesized from soil fungi found to be an efficient catalyst to degrade the hazardous dyes. Recent studies have reported longer time duration around 15 min for BB degradation by Iron nanoparticles (22). In the present study degradation of BB was achieved using a few drops of biogenic Au NPs in 2-3 sec. These results suggest that Au NPs also potentially useful for removal of environmental pollutants and degradation of organic pollutants. Therefore, green synthesis methods will play a significant role in the study and enhancement of the catalytic degradation of pollutant dye effluents in the environment.

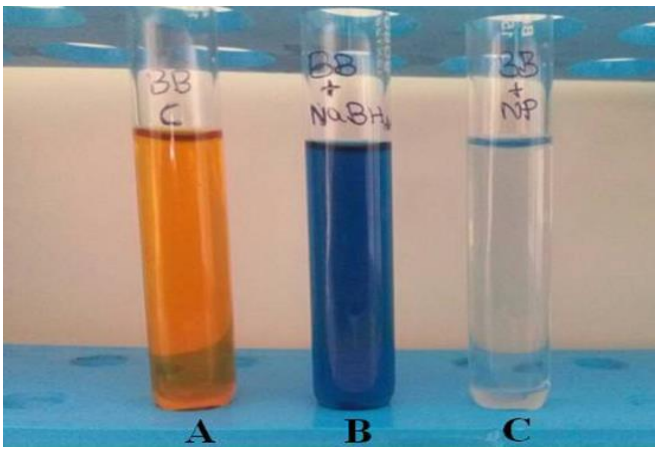

Fig .5: Bromo thymol blue dyedegradation by biogenic Au NPs.
A. Bromothymolblue, **B.** Bromothymolblue + $NaBH_4$, **C.** Bromothymolblue + $NaBH_4$ + Au NPs

CONCLUSION

A facile one step green synthesis method was developed to synthesize Au NPs successfully using fungi *Penicillium* sp. cell free extract without adding any external chemical agents. The fungal cell free extract act as both reducing and stabilizing agents during the synthesis of Au

NPs. The synthesis of Au NPs was confirmed by UV- visible peak obtained at 560 nm and size distribution was analysed by DLS studies. Further biogenic Au NPs showed very less and clinically acceptable level of haemolysis, this indicates Au NPs are biocompatible. Importantly, the as prepared Au NPs showed high catalytic activity for the degradation of bromothymol blue within a fraction of seconds after adding a few drops of Au NPs colloidal solutions.

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