J. Hum. Environ. Health Promot. 2016; 2(1):32- 38

Journal of Human, Environment and Health Promotion

Journal homepage: www.zums.ac.ir/jhehp

The Impact of Low Level X-rays on Biosynthesis of Gold Nanoparticles by Actinobacteria

Faranak Saghatchi [a,*], Gholam Ali Jafari [b], Jafar Taran [b], Zohre Farahmandkia [b]

[a] *Department of Radiology, School of Paramedical Sciences, Zanjan University of Medical Sciences, Zanjan, Iran.*
[b] *Department of Environmental Engineering, School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran.*

Corresponding author.** ***E-mail address: saghatchif@zums.ac.ir

ARTICLEINFO

Article history:
Received August 23, 2016
Accepted November 8, 2016

Article Type:
Original Article

Keywords:
Gold Nanoparticles
Biosynthesis
Radiation Hormesis
Actinomicetals

ABSTRACT

Background: Gold nanoparticles (GNPs) play an important role in medical, health and environmental applications. All kinds of microorganisms were found to be able to synthesize GNPs. The optimization of laboratory conditions for achieving more economical benefits of mass production has been studied widely.
Methods: This study assesses the enhancing effect of low-level X-rays on the biosynthesis of GNPs by Actinomycetals. The isolated Actinomycetes were grown aerobically in MGYP broth media. The harvested bacteria were suspended in 50 mL aqueous $HAuC_{l4}$ in 12 Erlenmeyer flask. Each group contained4 flasks. 2 groups of samples were irradiated by 30 mGy and 5 mGy X-rays respectively. The third group as control remained without irradiation. The solutions were shake- incubated for 120 h.
Results: After 5 days, the color of first group samples changed from milky to purple, while the color changing occurred after 10 days in the 2nd group samples and the control samples. The UV-vis absorption spectrometry of the irradiated aqueous medium by 30 mGy X-rays confirmed the formation of GNPs.
Conclusion: The findings showed that 30 mGy X-rays stimulated the microorganism to form GNPs in a half time in comparison to other groups.

1. Introduction

Nanotechnology and Nano sciences are gaining most attention due to their expected impact on many important fields such as medicine, health, and environment [1]. GNPs are the most known multi-functional nanoparticles for medical applications [2-5]. One of the major fields of application of nanoparticles in agriculture and the environment is detection and removal the chemical pollutants found in water, soil and air [6, 7].

Synthesis of metal nanoparticles apply various strategies such as physico-chemical methods and biological synthesis [8, 9]. Recent methods use biological processes or enzymatic reactions to reduce metal ions using special enzymes. This method has been successfully used in production of small nanoparticles on a large scale. Biological synthesis of nanoparticles is more reliable, eco-friendly and economical in terms of energy consumption [10].

To cite: Saghatchi F, Jafari GhA, Taran J, Farahmandkia Z. The Impact of Low Level X-rays on Biosynthesis of Gold Nanoparticles by Actinobacteria. *J Hum Environ Health Promot*. 2016; 2(1):32- 38.

Different types of microorganisms can produce gold nanoparticles in the form of intra or extra cellular [11-13]. The whole range of bacteria as well as Actinobacteria due to the ease of genetic manipulation, methods for industrialization and utilization of genetic engineering have attracted the most attention [11, 14-16]. Actinomycetals are a group of aerobic gram-positive bacteria, usually immovable and disciplinary. Actinomycetes can often be easily cultured in conventional environments. Actinomycetes in the soil remain mostly saprophytic and the pathogenic species are relatively low. Actinomycetes isolated from soil produce a variety of different antibiotics some of which are of great value such as streptomycin and neomycin due to their medicalsignificance [17].

Since the cellular mechanisms leading to the recovery of gold ions and the formation of gold nanoparticles are not well known, there is the potential for manipulation of key parameters that control cell growth and other activities to achieve optimal production of gold nanoparticles [18-21].

X-rays were discovered in 1895 by German physicist Wilhelm Conrad Roentgen. X-rays are electromagnetic waves, short wavelength and high energy photons [22].The radiation energy is transferred through the atoms and lead to the ionization in mater. Ionizing radiation has many Short-term and late hazardous effects [23].

However, over the past few decades, some scientists report of positive effects of low-level ionizing radiation named HORMESIS [24-27]. In 1980, Professor T.D Lucky reported the stimulating effects of ionizing radiation, both in animals and plants [28]. After the first report of this case, more than 3,000 articles were published about the positive effects of low- level ionizing radiation [29, 30]. Radiation Hormesis refers to generally positive physiological effects of low - radiation dose in the range of 1-50 cGy [26].

Several studies have demonstrated metabolism promotion in microorganisms, plants, invertebrates and laboratory animals by low-level ionizing radiation. These studies also have reported the effect of ionizing radiation on stimulation of respiratory system, boosting metabolism, cell resistance and extending life span [25, 31-34]. In various surveys many factors have been studied to increase production of nanoparticles by microorganisms [1, 13, 35-37].

So far, no studies have been conducted on the impact of ionizing radiation on optimization of biosynthesis of nanoparticles by microorganisms.

This study is the first research on this issue.

2. Materials and Methods

2.1. Materials

The absorption spectra of the samples were taken using a UV–vis spectrophotometer .

$HAuCl_4$ was obtained from Sigma–Aldrich, USA. All other chemicals such as agar, yeast extract, peptone, casein, starch, malt extract, nystatin, glucose, $CaCO_3$, $FeSO_4$, K_2HPO_4, KNO_3, $MgSO_4$ and NaCl were purchased from Merck, Germany. Freshly- prepared double distilled water was used throughout the experimental work.

2.2. Isolation and growth of Actinomycetes

The soil samples were collected from Angoran Lead mine in Zanjan. Five grams of sample was diluted by 9mL of distilled water. Then, 1ml of the aqueous solution was diluted again by 9 ml of distilled water and was shaken for 30 min. The aqueous solution was cultured in starch casein agar medium (starch 10 g, casein 0.3 g, $CaCO_3$ 0.02 g, $FeSO_4$ 0.01 g, K_2HPO_4 2 g, KNO_3 2 g, $MgSO_4$ 0.05 g, NaCl 2 g, and agar 15 g in 1,000 mL sterile distilled water at pH 7). To minimize the fungal and bacterial growth, nystatin (100 mg/L) was added to the cultures. The plates were then incubated at 28℃ for 7 days. After an initial period of bacterial growth in culture media, different colonies were obtained. In order to identify the bacteria genus, the colonies were identified based on typical properties such as chalky appearance surface color and the colonies smell like the smell of soil. Then the isolated colonies were cultured in medium containing starch casein agar plates and incubated for 7 days at 28 ℃. The Actinomycetes colonies were identified morphologically according to the procedures described in Bergey's manual of

determinative bacteriology. Fig. 1 shows the plates containing single clone of bacteria isolated from Angoran Mine. The samples were gram stain. The purple color of gram positive Actinomycetes were observed through a light microscope (Fig. 2). The isolated Actinomycetes were grown aerobically in 500 mL of MGYP broth media (malt extract 3 g, glucose 10 g, yeast cultures were incubated at 28 ℃ for 72 h with extract 3 g and peptone 5 g per in one litter of distilled water and pH 7. The continuous shaking (200 rpm).

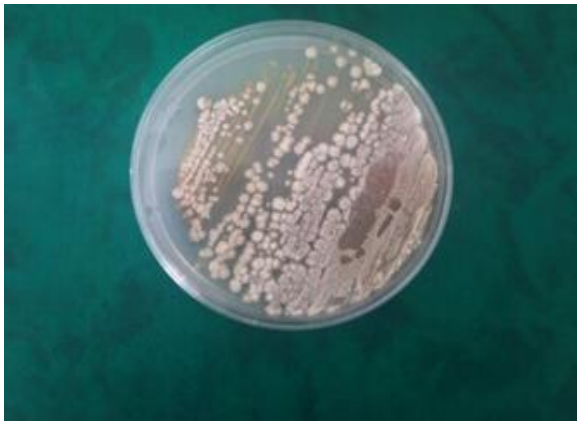

Fig. 1: Plates containing single clone of bacteria isolated from Angoran Mine.

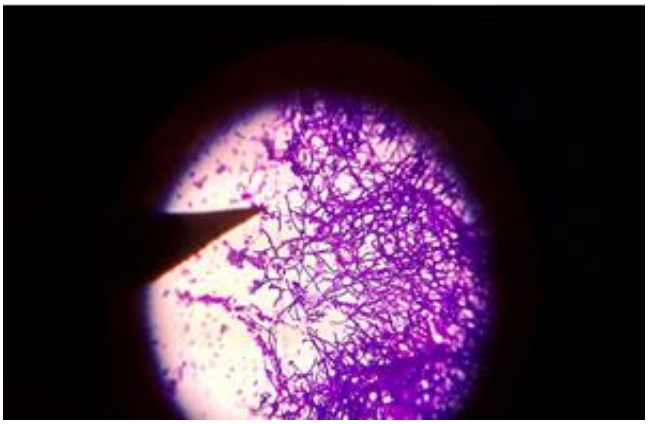

Fig. 2: Microscopic view of microorganisms isolated from Angoran Mine.

2.3. Irradiation and biosynthesis of GNPs

The cultures were then centrifuged at 6000 rpm for 20 min. The harvested bacteria were washed with distilled water under sterile condition. About 2 g of wet weight bacteria was suspended in 50 mL of 1 mM aqueous $HAuCl_4$ in each 12 Erlenmeyer flasks in 3 groups. Then the first group of samples (4 flasks) was irradiated to 30 mGy X-rays, by 200Kv Shimadzu X- ray tube while the second group (4 flasks) was irradiated to 5mGy X-rays. The control group (4 flasks) remained without radiation. All solutions were then shake- incubated (200 rpm) at 25℃ for 120 h.

2.4. Characterization of Gold Nanoparticles

The preliminary detection of the formation of GNPs was carried out by observing the color change of the solutions after irradiation to 30 mGy X-rays. The absorption spectra of the samples were taken using a UV-vis spectrophotometer from 300 to 800 nm (International MAX-USA, Genesys E5).

3. Results and Discussion

After 5 days the suspended bacteria to aqueous HAuCl4 which were irradiated to 30 mGy X-rays changed their color from milky to purple (Fig. 3).

This was the result of reduction of gold ions to GNPs. The UV-vis spectroscopy indicated that the samples had the maximum absorption at 540 nm (λ max of GNPs) attributable to the Surface Plasmon Resonance band (SPR) of gold nanoparticles [38]. Figure 4 shows the UV-vis spectra recorded from the irradiated bacteria to 30 mGy X-ray. The 5 mGy irradiated and control flasks remained unchanged. Only after 10 days of incubation the color change was observed in the control and 2nd group of samples. UV–vis spectroscopy demonstrated the reduction of the gold ions to GNPs in the second group of samples and the control group as well. Gold nanoparticles have a broad range of applications in a variety of fields such as medical diagnostics and treatment, industry and the environment [4]. The range of applications for gold nanoparticles is growing rapidly. Gold nanoparticles are used to detect biomarkers in the diagnosis of heart diseases, cancers, and infectious agents [16]. One of the major fields of application in agriculture and the environment is detection and cleanup of chemical pollutants found in water, soil and air [7].

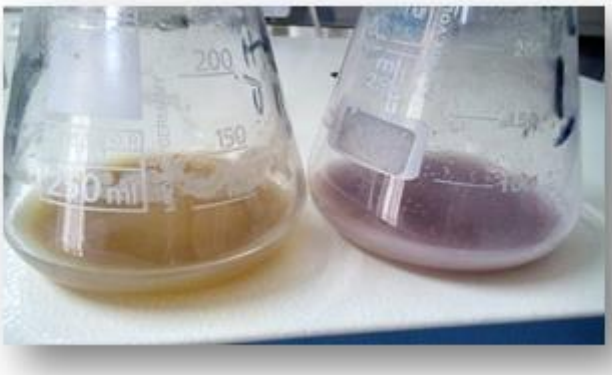

Fig. 3: The Color change in Actinomycetes irradiated to X-rays after 120 h incubation.

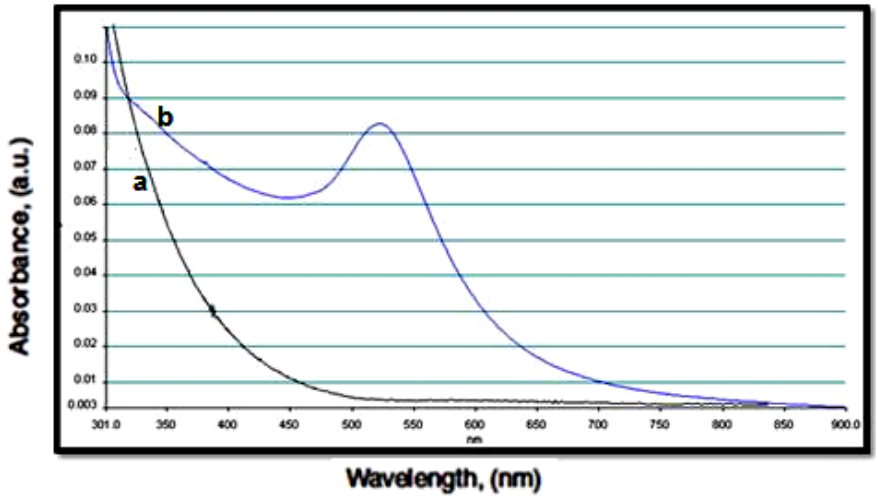

Fig. 4: UV- vis spectra recorded from the samples (a) before and (b) after synthesis of gold nanoparticles.

There are some methods to synthesize nano sized particles such as chemical synthesis and solid state processes. Biological synthesis of nanoparticles known as green synthesis is more reliable and eco-friendly. According to the effectiveness and flexibility, biological production systems are of particular value. Some single-celled organisms such as bacteria and algae are capable of synthesizing biocompatible nano particles [14].

In this regard manipulation of key parameters that control cell growth could lead us to achieve optimal production of gold nanoparticles. X-rays are electromagnetic waves short wavelength and high energy photons. Several studies, has demonstrated metabolism promotion in microorganisms, plants, invertebrates and laboratory animals by low-level ionizing radiation [25]. The goal of this study is the Impact of low level X-rays in the formation of GNPs by microorganisms. The colonies of microorganisms were collected from Lead mine in North West of Iran and characterized by microscopic and macroscopic studies as Actinomicetals. Different types of microorganisms as well as Actinobacteria can produce gold nanoparticles when exposed to $HAuCl_4$ solution [11, 14]. In various surveys many factors been studied to increase production of nanoparticles by microorganisms by changing the culture medium or the physical parameters [36, 37]. Our results emphasize the Hormetic effect of Ionizing radiation in microorganisms. Reduction

of the aqueous gold ions during exposure to 30 mGy X-rays may be easily followed by UV-vis spectroscopy. The earlier color change and UV–vis spectroscopy results in irradiated samples in first group reveals that X-rays stimulates the formation of gold nanoparticles by microorganisms. The late color change in 2th groups of samples could result of low intensity of X-rays that wouldn't impose sufficient stimulation to formation of GNPs in 120h. In future it is predictable that complementary studies in biosynthesis of GNPs by microorganisms under influence of Ionizing radiation and optimization of other laboratory conditions could be more applicative and affordable .

4. Conclusion

The findings showed that X-ray radiation stimulates the Actinobacteria to form gold nanoparticles.

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