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Biosynthesis and Optimization of Selenium Nanoparticles Using *Streptomyces sp*

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Abstract: In the present study, a bacterial strain isolated from a soil sample taken from the region around the root of the plant (Alhagi graecorum) that is growing in the desert west of the Sohag Governorate and molecularly identified as Streptomyces sp. was evaluated for the capability of selenium nanoparticles (SeNPs) production. The results showed that the bacterial strain could produce stable SeNPs sustainably within a week under optimized conditions (pH 7 and 32 °C) at its extra/intracellular wall. Subsequently, UV-Vis spectra were used to characterize SeNPs produced under different parameters, showing changes in the absorbance around 582 nm - 620 nm. The antimicrobial effects of SeNPs formed under different pH were also examined against pathogenic Escherichia coli. The results showed that SeNPs formed at acidic and basic pH induced substantially less antibacterial activity than those formed at a neutral pH where the diameter of the inhibition zone of SeNPs synthesized at pH 7 was 20 mm, but at pH 4 (13 mm), pH 5 (14 mm) and pH 9 (17 mm).
Keywords: Streptomyces sp, 16s rRNA, Selenium nanoparticles (SeNPs), Capping agent, Antimicrobial effect.

1. Introduction

Nanotechnology has emerged as one of the rising multi-disciplinary topics gaining worldwide interest and having a significant role in agriculture, the environment, and pharmaceuticals. Despite using numerous routes for nanoparticle synthesis, the incorporation of hazardous and expensive chemicals makes the process inadequate for continuous production. Thus, the need to develop a sustainable, environmental and eco-friendly approach has recently increased [1]. As a result of their greater surface-to-volume ratio and higher surface energy, nanoscale particles exhibit several distinct characteristics. Metal nanoparticles (MNPs), in particular, have numerous applications in diverse fields of physics, chemistry, biomedicine, and material sciences [2]. MNPs are considered the essential building blocks of nanotechnology and have a wide range of applications in areas like catalysis, optoelectronics, biological diagnostic probes, and display devices [3].

Physical and conventional chemical approaches [4,5] can both be used to produce nanoparticles. While chemical methods are the most used for creating nanoparticles, some chemical procedures cannot avoid using harmful substances during the synthesis phase. As prospective eco-friendly alternatives to chemical and physical processes, biological methods for making MNPs include microbes, enzymes, and plant or plant extract. Many biomimetic methods have been developed recently due to the growing interest in synthesizing nanoparticles using biological processes. Microorganisms, including bacteria, yeast, fungi, and algae, are crucial for recycling minerals in the environment. Some microbes can grow and live even in environments with high metal ion concentrations [6]. It is well-known that different organisms may generate biologically diverse nanoparticles with different physical characteristics [7-9].

By changing the metal ions' redox state, it is possible to reduce or even remove their toxicity; in some instances, this can result in the production of precise nanoscale particles [10]. Selenium (Se), a member of group 16 of the periodic table, is well-known for being an excellent semiconductor and photoelectric material. Additionally, Gao et al [11]. demonstrated the antioxidant qualities of selenium's hollow spherical nanoparticles. Wang et al [12] revealed similar findings that nano-Se could function as an antioxidant with a lower risk of selenium poisoning. Selenium nanoparticles' antimicrobial, antioxidant, anti-cancer, and biofilm inhibitory effects have recently been revealed. It is recognized that several factors influence the synthesis and shape of NPs in biological systems [13-15] There are several studies have been reported to control the size, shape, and morphology of gold and silver nanoparticles (NPs) [14,16] However, little research was done on SeNPs; therefore, systematic control is required to enhance SeNPs' overall features. Our study investigates SeNPs synthesis using the supernatant of Streptomyces culture and systematically identifies the factors affecting their biological and physicochemical characteristics during preparation.

Studies also examined antioxidant, antimicrobial, and anticancer effects. In the mitochondria of live cells, yellow MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide, a tetrazole) is converted to purple formazan. As this reduction occurs solely when the mitochondrial reductase enzymes are in operation, the conversion is directly correlated with the quantity of viable (alive) cells.

2. Materials and method

2.1. Isolation and screening of bacterial strains

A soil sample is taken from the region around the root of the plant (Alhagi graecorum) growing in the desert west of the Sohag Governorate (Dar El-Salam, Sohag, Egypt). To prevent the growth of bacteria and fungi, 0.1 g of soil was serially diluted and plated on starch casein agar (SCA) plates with nystatin (100 µg/ml) and nalidixic acid (20 µg/mL) amendments [17]. Following this, five actinobacterial strains were chosen based on their morphology and tested for the ability to produce SeNPs in a conical flask containing 5 g of each wet biomass suspended in a 100 ml aqueous solution of 1 mM Na_2SeO_3. Inoculated flasks were cultured in a shaking incubator (180 rpm) at 32 °C for 5-7 days, and the occurrence of a color shift from white to red in the media indicates the synthesis of SeNPs [18].

2.2. Identification of potential strain

Microscopic examination and biochemical tests were used for the preliminary identification of isolated bacterial strains as described in (Bergey's Manual) [19]. Further identification of bacterial strain was identified using the 16s rRNA gene sequence technique. DNA was isolated as described by [20]. With minor modifications to the previously standard approach, the phenol-chloroform extraction protocol was adapted for genomic DNA isolation from a potent actinobacterial strain [21].

The isolated DNA's quality and amount were confirmed using agarose gel electrophoresis. Using the primers 27F (5'AGTTTGATCCTGGCTCAG3') and 1492R (5'ACGGCTACCTTGTTACGACTT 3'), the 16S rRNA gene was annealed before being amplified by Taq polymerase. A partial sequence of 16S rRNA has been done by a micro gene company in Korea. . The BLASTN tool also calculated the degree of similarity between the obtained sequence and the reference sequence in the genomic database.

2.3. Biosynthesis of Selenium nanoparticles (SeNPs) using potential strain-E3

In preliminary screening, strain E3 was chosen and employed for the biogenic synthesis of SeNPs. After being added to an Erlenmeyer flask with 100 ml of nutrient broth, the actinobacterial inoculum of strain E3 was shaken at 180 rpm for 5-7 days. Centrifugation was used to extract the biomass from the cell supernatant for 30 minutes at 5000 rpm. An aqueous solution containing 100 mL of 1 mM Na_2SeO_3 was added to 5 g of wet biomass, weighed, rinsed three times with sterile deionized water, and then incubated for 72 hours in a rotator shaker [22]. Simultaneously, a culture medium not-containing bacterial cells and the supernatant containing 1mM of Na_2SeO_3 were used as positive and negative controls, respectively. Selenium nanoparticle generation was observed visually and confirmed spectroscopically (200 -800 nm).

2.4. Optimization of parameters for obtaining Selenium nanoparticles (SeNPs)

2.4.1. The effect of concentration of Na_2SeO_3

The effect of Sodium Selenite (Na_2SeO_3) concentrations on selenium reduction and nanoparticle synthesis was evaluated by incubating the test samples at various Na_2SeO_3 concentrations, including 0.5, 1, 2, 3,4, and 6 mM with pH 7 and incubated at 32°C, 180 rpm.

2.4.2. The effect of pH

To understand the impact of pH on the biosynthesis of SeNPs by strain E3, the pH of the media was adjusted to 2, 4, 5, 7, 9, and 12 using 0.1 N HCl and 1N NaOH solutions. Bacterial cells were incubated in the shaker (180 rpm) at 32°C and amended with 2 mM Na_2SeO_3 concentrations.

2.4.3. The effect of temperature

A crucial parameter is the incubation temperature. The cells were exposed to 23.0 mM Sodium Selenite (Na_2SeO_3) and incubated at various temperatures, namely, 10°C, 20°C, 32°C, 40°C, 50°C, and 60°C. UV-Visible spectra were used to characterize NPs [23].

2.5. Antibacterial activity of SeNPs synthesized under different pH conditions

According to [24], with a minor modification, the disc diffusion method (Kirby-Bauer testing) was used to evaluate the antibacterial activity of selenium nanoparticles. SeNPs were synthesized at different pH (pH 2, 4, 5, 7, 9, and 12). Following that, they were neutralized by adding NaOH or HNO_3 to measure the diameter of the inhibitory zone of SeNPs after an acidic or alkaline pH treatment. [25] Typically, 100 µl of each pathogen culture was transferred to individual Muller Hinton agar (MHA) plates using sterile swabs. SeNPs synthesized under different pH conditions were added to individual filter paper discs. Then, the plates were incubated at 37°C for 24 hours, and the zone of inhibition (ZOI) formed around the disc was measured.

3. Results and discussion

3.1. Isolation and screening of bacterial strains for the biosynthesis of selenium nanoparticles

In this work, five different bacterial strains were isolated from soil and initially identified as actinobacterial strains. Subsequently, these strains were screened and compared for their ability to induce uniformly sized and shaped selenium nanoparticles at their intra/extracellular. Among the five examined actinomyces strains, E3 showed the most prominent one to reduce selenium ions to selenium nanoparticles at its extracellular (Figure 1) [26]. Therefore, it was chosen here, in our study, for further investigations.

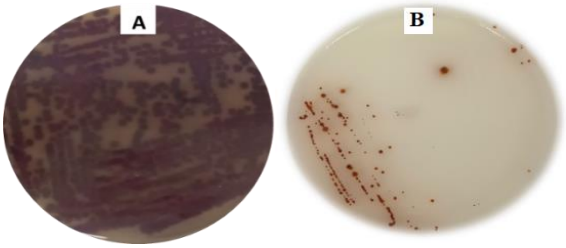

Figure 1. *Streptomyces sp* on starch casein agar (SCA) media (A) without Na_2SeO_3 and (B) with 2mM Na_2SeO_3.

3.2. Identification of Strain E3

Morphological characteristics of colonies were obtained based on microscopic examination. Strain E3 appears in branching mycelia. The substrate mycelium grows into the medium. Colonies on starch casein agar have a dark burgundy color (Figure 1). Strain-E3 was gram-positive, facultative aerobic, filamentous, non-motile, and forms conidia in chains from spore-bearing aerial hyphae. According to the differential biochemical characteristics, Strain E3 could utilize carbon compounds like D-glucose, D-mannitol, and fructose as a source of carbon and energy. It also utilized nitrogen sources like D-alanine, L-arginine, and L-tyrosine. Strain E3 was positive for catalase, oxidase, urease, and H_2S production. It was also positive for Gram staining and spore staining. The ideal temperature for growth is 32°C. The selected bacterial isolate Strain-E3 was identified According to the morphological, physiological, and biochemical characteristics (Table 1) and compared with those described in different identification schemes and literature based on Bergey's Manual of Systematic Bacteriology [27]. It was concluded that the selected bacterial isolate Strain-E3 belonged to the family Streptomycetaceae and genus Streptomyces. The bacterial strain was identified using biochemical tests and molecular analysis of the 16s rRNA gene. Based on the 16s rRNA gene's partial sequence, the strain-E3 was identified as *Streptomyces sp*. The partial sequence of the 16S ribosomal RNA gene was deposited in the gene bank (Figure 2). The BLASTN tool also calculated the degree of similarity between the obtained sequence and the reference sequence in the genomic database as a phylogenetic tree (Figure 3).

Figure 2. Partial sequence of 16s rRNA (ribosomal RNA) gene of *Streptomyces sp*. Strain-E3.

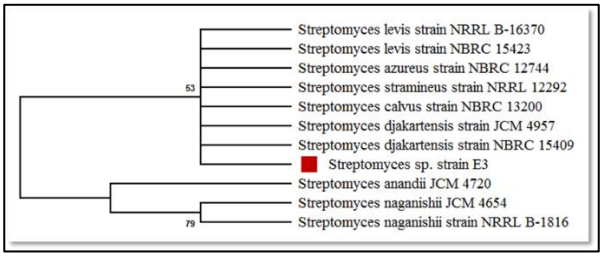

Figure 3. Neighbor-joining phylogenetic tree of strain E3 and related bacteria.

Table.1. Biochemical characteristics of *Streptomyces sp*.

Test	Result	Test	Result
Gram staining	+	Utilization of carbon source	
Spore staining	+	D-glucose	+
Catalase	+	D-mannitol	+
Oxidase	+	Fructose	+
Urease	+	Utilization of Nitrogen source	
H2S production	+	D-alanine	+
Indole	-	L-arginine	+
Voges-Proskauer(VP)	-	L-tyrosine	+

3.3. Biogenic synthesis of SeNPs using potential strain-E3

This work studied and reported the extracellular and intracellular production of selenium nanoparticles by the growth of Streptomyces sp. Visual inspection of the culture incubated with sodium selenite at 32°C for 5-7 days in the dark revealed a change in color from light yellow to bright red, indicating the production of red-colored elemental Se^0 (Figure 4, flask "C"), which are the distinctive feature for selenium nanoparticles [28]. However, no color change could be seen in the sodium selenite solution used as a negative control (Figure 5, Table 2). Control reactions in the absence of bacteria, with the culture medium, showed no color change or implied the requirement of bacteria for the bio-fabrication of SeNPs.

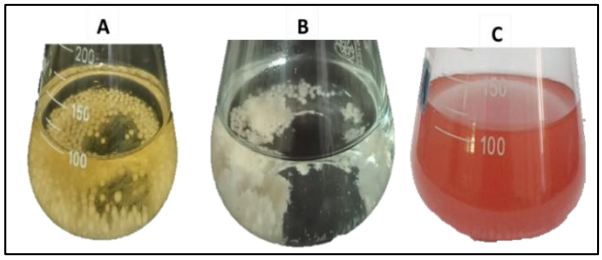

Figure 4. The digital photograph indicates the biosynthesis of selenium nanoparticles by *Streptomyces sp*. strain -E3. **(A)** *Streptomyces sp*. strain-E3 was grown in N.B broth for five days, **(B)** Bacterial strains were collected by centrifugation and incubated in deionized water amended with 2mM Na_2SeO_3 for five days, **(C)** selenium nanoparticles synthesis approved by appearances of the red color.

Figure 5. The photograph shows the red color that indicates SeNPs production (in the presence of bacteria) in tube **1** and no color change at negative control (in the absence of bacteria) in tube **2**.

3.4. Optimization of parameters for obtaining SeNPs

3.4.1. The effect of concentration of Na_2SeO_3

Na_2SeO_3 produced SeNPs at concentrations ranging from 0.5 to 6 mM. The color intensity of produced SeNPs increased with increasing Na_2SeO_3 concentration (Figure 6). With maximum synthesis observed at 2mM during absorption by UV-VIS (Figure 7).

3.4.2. The effect of pH

Formation of SeNPs was observed at tested pH (pH 4, 5, 7, and 9) but not observed at pH 2 and pH 12 (Figure 8). At 2mM Na_2SeO_3, the concentration biogenesis of SeNPs increased with the increase in pH up to 9. The spectra were altered because of the variable shape of SeNPs with maximum synthesis observed at pH 7 during absorption by UV-VIS (Figure 9).

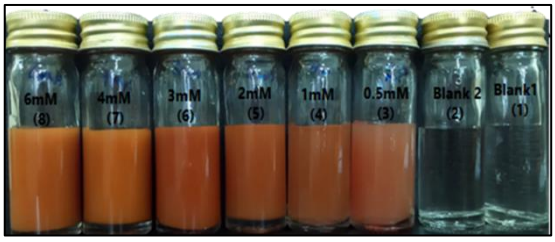

Figure 6. Digital photograph of test tubes (3-8) containing the bacteria *Streptomyces sp* culture of reactions with different concentrations of an aqueous solution of Na_2SeO_3 (0.5mM, 1mM, 2mM, 3mM, 4mM, and 6mM) for 5-7dayes at 32°C, pH 7.0, tube (1-2) the corresponding blank reactions.

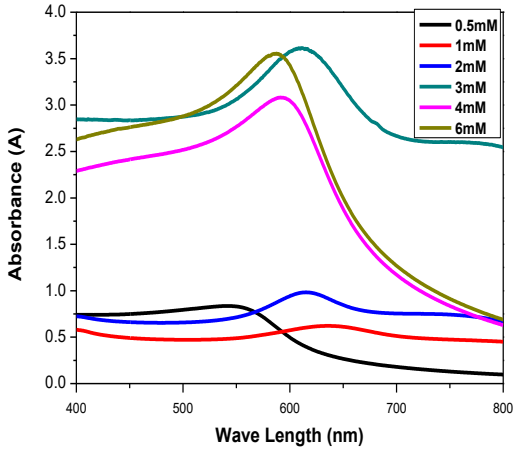

Figure 7. Spectra of UV-Vis absorption of selenium nanoparticles solution after the reaction of different concentrations of aqueous Na_2SeO_3 solution at neutral pH with the bacteria *Streptomyces sp* culture for 5-7 days.

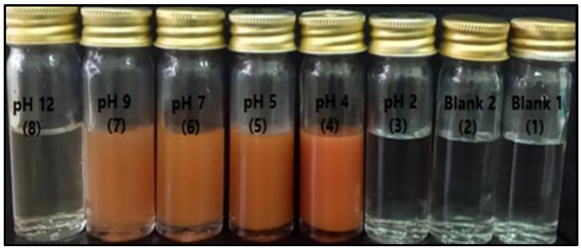

Figure 8. The synthesis of selenium nanoparticles is indicated by the various colors produced from the selenium reduction assay at various pH levels. The tubes with numbers (1-2) represent blank reactions, whereas those with even numbers (3-8) represent *Streptomyces sp* culture reactions with 2mM of Se_2NaO_3 at pH 2, 4, 5, 7,9, and 12 accordingly.

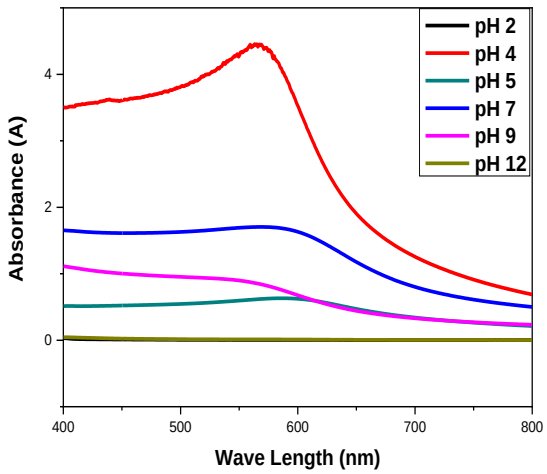

Figure 9. According to the surface Plasmon resonance, UV-Vis absorption spectra acquired for the various pH values, selenium nanoparticle formation is indicated by a change in the absorbance around 582 nm.

3.4.3. The effect of temperature

Shape and size-altering agents include temperature and pH. [29] SeNPs were synthesized at 32°C, 40°C, and 50°C (Figure 10), with the highest levels of synthesis seen at 32°C (Figure 11). Our findings are consistent with those of Lortie et al., who could not synthesize SeNPs at higher temperatures [29]. This is likely because the proteins or biomolecules necessary for reducing sodium selenite are only active at 32°C, 40°C, and 50°C. Therefore, 32°C was chosen as the ideal temperature.

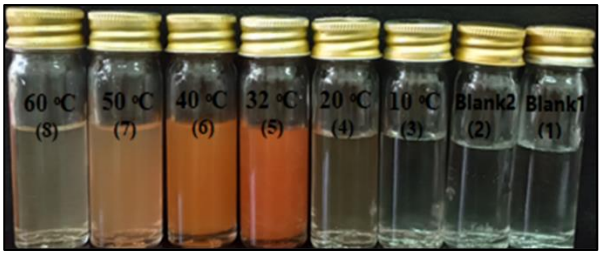

Figure 10. The synthesis of selenium nanoparticles is indicated by the various colors acquired from the selenium reduction assay at various temperatures. The numbers (1-2) represent the corresponding blank reactions, while the numbers (3–8) represent *Streptomyces sp* culture reactions with 2 mM Na_2SeO_3 at pH 7, incubation at various temperatures as specified (10, 20, 32, 40, 50, and 60 °C) respectively.

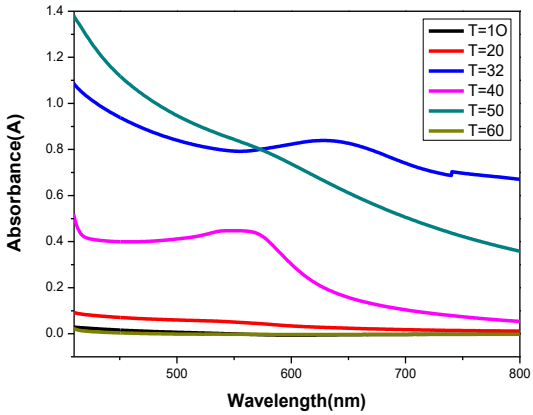

Figure 11. Selenium nanoparticle production may be seen in the UV-Vis absorption spectra of the surface Plasmon resonance recorded for the various temperatures as a change in the absorbance around 582 nm - 650 nm.

3.5. Antibacterial activity of SeNPs synthesized under different pH conditions

SeNPs treated with different pHs ranging from acidic pH 2 to alkaline pH 12 exhibited different antibacterial effects than the control SeNPs at pH 7 (Table 2). This procedure aimed to determine the influences of pH on the antibacterial activity of produced SeNPs against strains of pathogenic bacteria sensitive to SeNPs. The SeNPs produced at acidic pH (pH 4 and 5) exhibited an antibacterial effect lower than the SeNPs synthesized at neutral pH (Figure 12). The diameter of the inhibition zone of SeNPs synthesized at pH 4 against *Escherichia coli* decreased to (13.5 mm), compared with SeNPs at pH 7 (20 mm). In contrast, the inhibition zone decreased to (17mm) when produced at pH 9 compared with the SeNPs at pH 7 (20mm). These observations unequivocally show that, compared with SeNPs produced at neutral pH, SeNPs produced at an acidic pH induced substantially less antibacterial activity. When the pH decreased, the antimicrobial activity decreased too. The reasons may refer to the ability to low pH to destroy the protein capping agent of SeNPs, which has the crucial function of antimicrobial activity. Our results are in good agreement with [30]. The report investigated the antimicrobial effects of silver nanoparticles synthesized under different conditions and reported that the characteristics of NPs synthesized under acidic pH were changed, causing aggregation. This resulted in NPs having a considerably harder time anchoring and being unable to penetrate through bacterial membranes, sharply decreasing their antibacterial activity. The antibacterial activity of SeNPs synthesized at pH 9 was slightly decreased from neutral SeNPs, as shown in (Table 2). However, the antibacterial activities of synthesized SeNPs decreased after acid or alkaline pH. Thus, to effectively use antibacterial materials in their intended applications, it is crucial to avoid employing them under the mentioned circumstances.

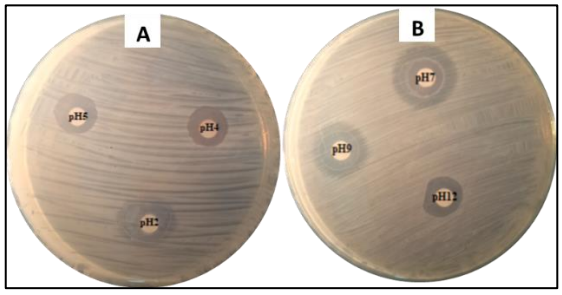

Figure 12. Photographs show the antibacterial activity of SeNPs synthesized by strain.E3 under different pH conditions against *Escherichia coli* (A) and (B).

Table 2. Sizes of the inhibition zones (mm) of SeNPs produced under different pH conditions against *Escherichia coli.*

Diameter of inhibition zone in (mm)				
Pathogen	SeNPs at pH 4	SeNPs at pH 5	SeNPs at pH 7	SeNPs at pH 9
Escherichia coli	13.5 mm	14 mm	20 mm	17mm

4. References

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