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Screening of Soil actinomycetes ability to biosynthesis Zinc oxide nanoparticles

Zeinab N. Abdelhafez[1], Ahmed A. Moubarak[1], Hamed M. El-Shora[2] , Ahmed A. Hamed [3*] and Mervat G. Hassan[1]

[1]*Botany and Microbiology Department, Faculty of Science, Benha University, Banha, Qalyubia, Egypt.*
[2] *Botany Department, Faculty of Science, Mansoura University, Mansoura, Dakahlia, Egypt.*
[3] *Microbial Chemistry Department, National Research Centre, 33 El-Buhouth Street, Giza, Dokki, Egypt.*
E-Mail: zeinebna@gmail.com

Abstract

The study aimed to explore the biotechnological potential of actinomycetes in the synthesis of zinc oxide nanoparticles (ZnONPs) through the assessment of their ability to form a white precipitate and the detection of ultraviolet (UV) peaks. Twenty actinomycete isolates were screened, and their responses were evaluated based on the intensity of white precipitate formation (+++ for intense, + for mild, - for absent) and the presence or absence of UV peaks related to ZnONPs. The results revealed diverse behaviors among the isolates, with notable variations in both white precipitate formation and UV responses. Isolates like S13, S17, and S292 demonstrated intense white precipitate formation along with positive UV responses, suggesting a potential relationship between their ability to reduce zinc ions and ZnONP synthesis. However, isolates such as S392 and S41, despite forming intense white precipitates, displayed negative UV responses, indicating the presence of additional factors influencing ZnONP formation. These findings underscore the complexity of the biochemical pathways involved in metal ion reduction and nanoparticle synthesis by actinomycetes. The study provides valuable insights into the biotechnological applications of actinomycetes in nanomaterial synthesis and environmental remediation. Further investigations into the underlying mechanisms can enhance our understanding and pave the way for sustainable and eco-friendly technologies.

Keywords: Zinc oxide nanoparticles, Biosynthesis, Actinomycetes, Isolation, Nanotechnology.

Introduction

In the field of material science, nanoscience and nanotechnology stand out as areas of research and growth. Researchers focus on creating materials at the nanoscale (1–100 nm) for a range of applications. Nanoparticles (NPs) are clusters of atoms, with properties such as surface characteristics, size on a quantum-level crystal structure, electric charge, shape, optical properties, distribution pattern, zeta potential, and heat conductivity. These features make them suitable for use in medical applications. A key aspect of nanotechnology is the synthesis of nanoparticles, which is typically done using chemical or biological methods that can involve conditions like high temperatures and pressures along with the use of large amounts of harmful chemicals. This can have impacts on the environment. Lead to less stable NPs. Therefore, it is important to explore fabrication techniques for NPs that are safe to use, eco-friendly, and easily scalable. As a result, there is a growing interest in adopting environmentally friendly approaches, in nanotechnology that utilize toxic substances and gentle processes **(Shah et al., 2020; El-Belely et al., 2021**; **Abdelkader et al.; 2022; Lal et al., 2022).**

The concept known as " nanotechnology" refers to an eco-way of producing nanomaterials by reducing or eliminating the use of hazardous materials [Citation6]. Biological synthesis of NPs, utilizing green chemistry as an eco-friendly approach, involves active compounds from various biological entities such as plants, fungi, actinomycetes, yeast, bacteria, algae, and cyanobacteria **(Salem and Fouda, 2021)**. Specifically, endophytic fungi prove more suitable for NP synthesis due to their ability to produce substantial biomass resistant to flow pressure and agitation, rapid growth, secretion of large enzyme quantities, and ease of handling in downstream processing. This simplifies the fabrication of NPs for immediate use in diverse applications **(Singh et al., 2017).** Endophytes, residing within healthy plant tissues, possess distinctive traits and exhibit potential in synthesizing antibacterial, antiviral, antioxidant, insulin-mimicking, and immunosuppressive activities **(Singh et al., 2017; Clarance et al.,2020).** Bioactive constituents synthesized by living organisms show significant potential in treating various diseases, including heart disease, diabetes, cancer, and infectious diseases **(Imran et al., 2021).**

Zinc, a vital nutrient for humans and animals, plays a crucial role in DNA replication, repair, oxidative stress, and cell cycle progression. Zinc oxide nanoparticles (ZnONPs), compared to traditional zinc sources, exhibit lower toxicity, increased absorption rates, higher bioavailability, immune system enhancement, superior biocompatibility, and act as potent antimicrobial agents **(Anwar et al., 2022; Hatab et al., 2022).** ZnONPs have gained widespread attention among metal oxide NPs due to their unique characteristics, including electro-optical and chemical properties that can be altered by changing NP morphology. Additionally, they provide UV protection, electrical conductivity, and photocatalytic activity, expanding their range of applications **(Mohamed et al., 2021; Lal et al., 2022).**

1. Collection of Soil Samples

Soil samples were gathered from various locations in Benha, Egypt, coded, and stored in falcon tubes. These were then transported to the microbiology lab at the National Research Center under standard conditions. In the lab, 0.5 g of each soil sample was added to a test tube with 10 ml of sterile water and shaken for 10 minutes at 27 °C using an orbital shaker incubator **(Casida, 1984; Haque et al., 1992).**

2. Isolation of Actinomycetes

Actinomycetes were isolated from soil samples using the serial dilution technique. Briefly, 0.5 g of each sample was mixed with 9 ml of distilled water in separate test tubes and vigorously shaken. These tubes served as stock cultures, and from each stock, 1 ml aliquots were aseptically transferred to test tubes with 9 ml of sterile physiological saline. Further dilutions were made, and 0.1 ml of each sample was plated on a starch nitrate agar medium. After incubating for 7 days at 30°C, actinomycetes colonies with distinct morphology

Material and methods

were selected, purified using the streaking method, and labeled S1, S2, ... S9. Purified strains were stored at 4°C on starch nitrate slants **(Yaminisudha et al., 2015).**

3. Screening of Isolated Actinobacteria for Biosynthesis of Zinc Oxide Nanoparticles

To evaluate the ability of isolated actinomycetes to biosynthesize ZnO nanoparticles (ZnONPs), the strains were cultured in 250 ml flasks with 50 ml of ISP2 media. After seven days of incubation at 30-32°C, the cultures were centrifuged to separate the supernatants. For ZnO NPs synthesis, a solution of zinc acetate (0.1 M) and sodium hydroxide solution (0.4 M) was combined in a 50 mL flask. Additionally, 50 mL of actinomycetes culture was added, and the mixture was shaken at 40°C for 15 minutes. The flask was then heated in a microwave oven for 1-2 minutes and allowed to cool for 1 hour. The appearance of white color deposits confirmed the successful formation of ZnO nanoparticles in a powdery form **(Mishra et al., 2013).**

Results

1. Sample collection.

Soil samples were collected from different locations in Benha. The samples were coded according to the site of collection, the following table shows the place of collection and the assigned code for each sample.

Table (1) A datasheet of all collected samples

Serial	Location	Sample code
1	Area 1	S1
2	Area 2	S2
3	Area 3	S3
4	Area 4	S4

Fig. (1) Live images from different locations at Benha

2. Isolation of actinomycetes from the collected soil samples

Isolation of actinomycetes from collected soil samples was carried out using serial dilution methods. Twenty isolates were obtained from the collected soil samples. In **Table 2** of isolated actinomycetes, a diverse range of surface colony colors and pigmentation is observed, reflecting the potential genetic and biochemical variations among the isolates. The color variations, such as grey, white, green, pink, and yellow, indicate the presence of different pigments produced by these actinomycetes. Pigmentation in actinomycetes is often associated with the production of secondary metabolites, which can have various biological activities. The grey and greyish-white colonies (isolates S16, S292, S18, S293, S17, and S43) suggest a similar color palette, potentially indicating shared biochemical pathways or genetic traits among these isolates. White colonies (isolates S31, S38, S11, S27, and S13) also exhibit diversity in pigmentation, with shades ranging from yellow to light orange. Notably, the isolate S37 with pink pigmentation stands out, indicating the potential production of unique secondary metabolites.

The presence of green colonies in isolates S392, S33, and S16 suggests the synthesis of specific pigments, possibly related to antibacterial or antifungal activities. Yellowish and light green colonies (isolates S47, S291, S39, and S12) add further diversity to the color spectrum, highlighting the potential richness of secondary metabolites produced by these actinomycetes.

Table (2) Isolated actinomycetes morphology and pigment formation

No.	Code of isolate	Surface colony color	Pigmentation
1.	*S16*	grey	Grey
2.	*S31*	white	*Yellow*
3.	*S292*	grey	Grey
4.	*S18*	greyish white	*Yellow*
5.	*S38*	white	*Yellow*
6.	*S293*	grey	Grey
7.	*S11*	white	*Light orange*
8.	*S17*	grey	Grey
9.	*S47*	*yellowish white*	*Light green*
10.	*S392*	*Dark green*	*Green*
11.	*S37*	*pink*	*Pink*
12.	*S291*	*yellow*	*Yellow*
13.	*S41*	*yellowish*	*Yellow*
14.	*S27*	white	*Yellow*
15.	*S13*	white	White
16.	*S33*	*green*	*Green*
17.	*S39*	*yellowish*	*Yellow*
18.	*S43*	grey	*Yellow*
19.	*S12*	Light grey	*Yellow*
20.	*S16*	*green*	*Green*

Fig. (2) Morphology of the actinomycete colonies

Table (3) Formation of ZnONPs based on UV–Vis absorption spectrum and white precipitate formation.

No.	Code of isolate	*White ppt.*	*UV*
1.	S13	+++	+ve
2.	S392	+++	-ve
3.	S41	+	-ve
4.	S27	-	+ve
5.	S31	-	-ve
6.	S37	-	-ve
7.	S17	+++	+ve
8.	S39	-	-ve
9.	S18	-	+ve
10.	S43	-	-ve
11.	S292	++	+ve
12.	S12	-	-ve
13.	S38	-	+ve

3. Screening the isolated actinomycetes filtrate ability to the biosynthesis of ZnONPs

The screening of actinomycetes for their ability to form a white precipitate (white ppt) and the ultraviolet detection of peaks of ZnONPs represents a crucial step in understanding their potential bioremediation and nanomaterial synthesis capabilities. The results obtained from Table 3 highlight the diverse responses of the actinomycete isolates to these screening parameters.

Firstly, the formation of a white precipitate is indicative of the actinomycetes' ability to reduce metal ions and participate in the synthesis of metal nanoparticles. In this context, isolates S13, S392, S17, and S292 exhibited significant white precipitate formation (+++), suggesting a high potential for mediating the reduction of zinc ions. The varying intensity of white precipitates among these isolates could be attributed to differences in their enzymatic activities or metabolic pathways involved in metal reduction.

Secondly, the ultraviolet detection of peaks for ZnONPs showed both positive (+ve) and negative (-ve) results. Isolates S13, S27, S17, and S292 exhibited a positive UV response, indicating the presence of peaks associated with the formation of ZnONPs. Conversely, isolates S392, S41, S31, S37, S39, S18, and S43 showed a negative UV response, suggesting a lack of peaks associated with ZnONPs. This variation in UV responses could be linked to differences in the actinomycetes' enzymatic machinery involved in the synthesis of ZnONPs.

The comparison of white precipitate formation and UV responses reveals some interesting patterns. Isolates S13, S17, and S292, which showed intense white precipitates, also exhibited positive UV responses, suggesting a potential correlation between their ability to form white precipitates and synthesize ZnONPs. On the other hand, isolates like S392 and S41, which displayed intense white precipitates, showed negative UV responses, indicating that other factors may influence the formation of ZnONPs.

Discussion

Metal oxide nanomaterials have been used in cosmetics, paints, plastics, and textiles because of their antimicrobial ability (Azam et al., 2012). Among various inorganic metal oxide nanomaterials, zinc oxide (ZnO) is one of the most widely used as an antimicrobial agent, because of its Zn presence, which is an essential mineral element to humans, and its effective activity (Doumbia et al., 2015). ZnO NPs are recognized as safe or GRAS, nontoxic, and bio-compatible particles. The biological method for the synthesis of ZnO NPs is good as it is a simple method (Zhang et al.,2013).

Actinomycetes are gram-positive bacteria, distinguished by their non-motility and similar morphology to fungi (Bahrulolum et al., 2021). Actinomycetes also produce enzymes, vitamins, and pigments that can be put to other uses. The biosynthesis of Zinc oxide nanoparticles from actinomycetes involves utilizing their inherent enzymatic machinery to reduce and stabilize metal ions into nanoparticles. (Pooja et al., 2015; Ravi and Vasantba, 2016; Orooba et al., 2017; Rotich et al., 2017).

Actinomycetes colonies were isolated by serial dilution method using starch casein nitrate agar medium. In the present study, 20 different actinomycetes were isolated from the soil sample (Gurung et al.; 2009). The color variations, such as grey, white, green, pink, and yellow, indicate the presence of different pigments produced by these actinomycetes. Pigmentation in actinomycetes is often associated with the production of secondary metabolites, which can have various biological activities (Parmar et al.; 2016). The white precipitate and UV–Vis absorption spectrum were formed to indicate the synthesis of ZnO nanoparticles. the formation of a white precipitate is indicative of the actinomycetes' ability to reduce metal ions and participate in the synthesis of metal nanoparticles. The varying intensity of white precipitates among these isolates could be attributed to differences in their enzymatic activities or metabolic pathways involved in metal reduction. the ultraviolet detection of peaks for ZnONPs showed both positive (+ve) and negative (-ve) results. This variation in UV responses could be linked to differences in the actinomycetes' enzymatic machinery involved in the synthesis of ZnONPs (Ibrahem et al., (2017)

Conclusion

In conclusion, the screening of actinomycete isolates for their ability to form a white precipitate and the ultraviolet detection of peaks related to ZnONPs reveals a diverse array of responses among the isolates. The isolates, such as S13, S17, and S292, exhibiting intense white precipitate formation, also display positive UV responses, suggesting a potential correlation between their ability to reduce zinc ions and synthesize ZnONPs. On the other hand, isolates like S392 and S41, despite displaying intense white precipitates, show negative UV responses, indicating additional factors influencing the formation of ZnONPs. These findings underscore the complexity of the biochemical pathways involved in metal ion reduction and nanoparticle synthesis by actinomycetes.

The observed variations in the screening parameters highlight the need for further investigation into the enzymatic machinery and metabolic pathways of the actinomycete isolates. Such detailed studies could unravel the specific mechanisms behind their ability to form white precipitates and synthesize ZnONPs. Understanding these processes is crucial for harnessing the biotechnological potential of actinomycetes in applications related to nanomaterial synthesis and environmental remediation.

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