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Phenotypic Characterization And Potential Of Endophytic Bacteria From Dahlia Tuber As Bioreducing Agents In Silver Nanoparticle (AgNPs) Synthesis

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Abstract:
Endophytic bacteria from Dahlia tubers have potential as bioreducing agents in the synthesis of silver nanoparticles (AgNPs). This study utilized six isolates of endophytic bacteria from Dahlia tubers (BEUD) to evaluate colony morphology, Gram characteristics, catalase activity, and the ability to reduce Ag^+ ions into AgNPs. Macroscopic observations showed variations in colony shape, margin, and elevation, while microscopic analysis revealed two Gram-positive and four Gram-negative isolates, with all isolates exhibiting positive catalase activity. The bioreduction screening indicated AgNP formation within the wavelength range of 404–429 nm, with the highest absorbance observed in BEUD2 (429 nm; 2.627), indicating the most effective silver ion reduction capacity. The bioreduction activity varied depending on the secondary metabolites produced by each isolate, including reductase enzymes, proteins, and phenolic compounds. BEUD2 was selected as the most promising candidate for further optimization. These findings suggest that endophytic bacteria from Dahlia tubers are a potential biological resource for the eco-friendly biosynthesis of AgNPs with antibacterial applications
Keywords: *Endophytic bacteria, Dahlia tuber, Silver nanoparticles, Bioreductor, Antibacterial*

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I. Introduction

Antibacterial resistance is a growing global concern and poses a serious threat to public health. This phenomenon is driven by the widespread and often uncontrolled use of synthetic antibacterial agents, including antibiotics, disinfectants, and biocides, across various sectors such as healthcare, industry, and agriculture. The excessive and irrational use of these agents creates continuous selective pressure, accelerating the emergence of resistant microorganisms [1]. In response to this issue, alternative approaches have been increasingly explored, one of which is the utilization of biological resources for nanoparticle synthesis through biosynthesis. This approach is considered more environmentally friendly, efficient, and sustainable compared to conventional chemical methods, as it exploits the biological ability of microorganisms to reduce metal ions into nanoparticles [2].

One plant with promising potential is Dahlia, which is known to possess tubers rich in secondary metabolites such as alkaloids, flavonoids, and saponins with bioactive properties. These compounds contribute to various biological activities, including antibacterial and antioxidant effects [3]. Additionally, dahlia tuber tissues serve as a habitat for endophytic bacteria capable of producing diverse bioactive compounds, such as phenolics, proteins, and reductase enzymes. These compounds play a role in metal ion reduction and nanoparticle formation, while also exhibiting antimicrobial activity against various pathogens [4]. Therefore, endophytic bacteria from dahlia tubers have strong potential as bioreducing agents in the synthesis of silver nanoparticles (AgNPs) with antibacterial properties.

Recent studies have demonstrated that endophytes, such as the endophytic fungus Aspergillus hiratsukae, are capable of synthesizing stable AgNPs with significant antimicrobial activity [5]. Other studies have reported that biosynthesis of AgNPs using endophytic microorganisms, including bacteria and fungi, produces nanoparticles that are effective in inhibiting the growth of pathogenic and even resistant bacteria [6]. Therefore, this study aims to explore the potential of endophytic bacteria isolated from dahlia tubers as bioreducing agents in the synthesis of silver nanoparticles (AgNPs).

II. Materials And Methods

Materials

The materials used in this study included endophytic bacterial isolates from Dahlia tubers (BEUD), Nutrient Agar (NA) medium, antibiotic media, crystal violet, safranin solution, 70% ethanol, 3% H_2O_2, 0.85% NaOCl, 0.5 McFarland standard solution, distilled water, and $AgNO_3$.

Macroscopic and Microscopic Identification

Bacterial identification was carried out through microbiological assays based on macroscopic and microscopic morphological observations. Macroscopic observation was performed visually to evaluate colony characteristics, including color, shape, margin, and elevation. Microscopic observation was conducted on bacterial preparations stained using the Gram staining method to determine cell shape and Gram characteristics, and observed under a light microscope at 1000× magnification using immersion oil. In addition, a catalase test was performed to determine the respiratory type of the bacteria, whether aerobic or anaerobic.

Screening of Endophytic Bacteria as Bioreductors

The screening of endophytic bacteria from Dahlia tubers as bioreducing agents was conducted to evaluate the potential of the isolates in the synthesis of silver nanoparticles (AgNPs), following the protocol of Sarina et al. (2020). Six bacterial isolates were used, and cultures grown in Nutrient Broth (NB) were centrifuged at 8,000 rpm for 15 minutes to obtain the supernatant. Subsequently, 5 mL of supernatant was mixed with 15 mL of 3 mM $AgNO_3$ solution in a 50 mL Erlenmeyer flask, then incubated at 37°C with a rotary shaker at 150 rpm under dark conditions for 72 hours. The formation of AgNPs was indicated by a color change of the solution from yellow to brown, which was further confirmed by measuring absorbance using UV–Vis spectrophotometry in the wavelength range of 300–800 nm.

III. Results

Macroscopic Identification

The isolation of endophytic bacteria from Dahlia tubers conducted by previous researchers resulted in six collected isolates. All isolates were subsequently coded as endophytic bacteria from Dahlia tubers (BEUD) and used in this study as test samples. Initial identification was carried out through macroscopic observation to evaluate colony morphological characteristics, including color, shape, margin, and elevation, as presented in Table 1

Tabel 1. Identification of Endophytic Bacterial Isolates from Dahlia Tubers (BEUD).

Isolate	Color	Shape	Margin	Elevation
BEUD 1	Yellowish white	Filamentous	Entire	Flat
BEUD 2	Yellowish white	Irregular	Serrated	Flat
BEUD 3	Yellowish white	Irregular	Undulate	Flat
BEUD 4	Yellowish white	Filamentous	Entire	Flat
BEUD 5	Yellowish white	Filamentous	Serrated	Flat
BEUD 6	Yellowish white	Filamentous	Serrated	Flat

The macroscopic observations of six endophytic bacterial isolates from Dahlia tubers (BEUD) revealed variations in colony morphology. All isolates exhibited a cream color and flat elevation, but differed in colony shape and margin. Isolates BEUD1, BEUD4, BEUD5, and BEUD6 were generally filamentous, with variations in margins ranging from entire to serrated, while BEUD2 and BEUD3 showed irregular shapes with serrated and undulate margins. This variation indicates phenotypic diversity among isolates, even though they originated from the same source.

These macroscopic characteristics can serve as preliminary parameters for distinguishing endophytic bacterial isolates prior to further identification through microscopic or molecular analyses [7]. Moreover, differences in colony morphology may reflect variations in metabolic capabilities and biological functions of each isolate.

Microscopic Identification

The results of microscopic analysis of six endophytic bacterial isolates from Dahlia tubers (BEUD), including Gram characteristics and catalase activity, are presented in Table 2.

Tabel 2. Identification of Endophytic Bacterial Isolates from Dahlia Tubers (BEUD).

Isolatate	Gram	Cell Shape	Catalase
BEUD 1	+	Rod	+
BEUD 2	-	Rod	+
BEUD 3	-	Rod	+

BEUD 4	-	Rod	+
BEUD 5	+	Rod	+
BEUD 6	-	Rod	+

The microscopic analysis of six endophytic bacterial isolates from Dahlia tubers (BEUD) revealed variation in Gram characteristics. Two isolates (BEUD1 and BEUD5) were classified as Gram-positive, while the other four isolates (BEUD2, BEUD3, BEUD4, and BEUD6) were Gram-negative. This variation indicates that the endophytic bacterial community in Dahlia tubers consists of diverse groups, including both Gram-positive and Gram-negative bacteria.

All isolates showed positive catalase activity, indicating their ability to decompose hydrogen peroxide (H_2O_2) into water and oxygen as a protective mechanism against oxidative stress. This characteristic supports the adaptability of endophytic bacteria in the dynamic host environment. These findings are consistent with previous studies reporting that catalase activity and Gram variation are important parameters in identifying the diversity of endophytic bacteria prior to further analysis [8].

Screening of Endophytic Bacteria as Biological Control Agents Against Plant Pathogens

Tabel 3. Screening of Endophytic Bacteria with Potential as Bioreductors

	Inhibition Activity (mm)	
Isolatate	**Wavelength**	**Absorbance**
BEUD 1	419	1,210
BEUD 2	429	2,627
BEUD 3	404	1,500
BEUD 4	412	1,401
BEUD 5	415	2,470
BEUD 6	421	1,211

The results showed that six endophytic bacterial isolates from Dahlia pinnata tubers exhibited different bioreduction abilities in synthesizing silver nanoparticles (AgNPs), as indicated by variations in absorption wavelengths (400–500 nm), which are characteristic of surface plasmon resonance. Isolate BEUD2 demonstrated the highest activity (429 nm; 2.627), followed by BEUD5 (2.470), while the other isolates showed relatively lower values.

These differences indicate that bioreduction capacity is influenced by the composition of secondary metabolites produced by each isolate, such as reductase enzymes, proteins, and phenolic compounds that play roles in the reduction and stabilization of nanoparticles. These findings are consistent with previous studies reporting that microbial bioreductive activity is highly dependent on the type of isolate and the extracellular metabolites produced [9]. Based on these results, isolate BEUD2 was selected as the best candidate for further optimization due to its highest silver ion reduction capability.

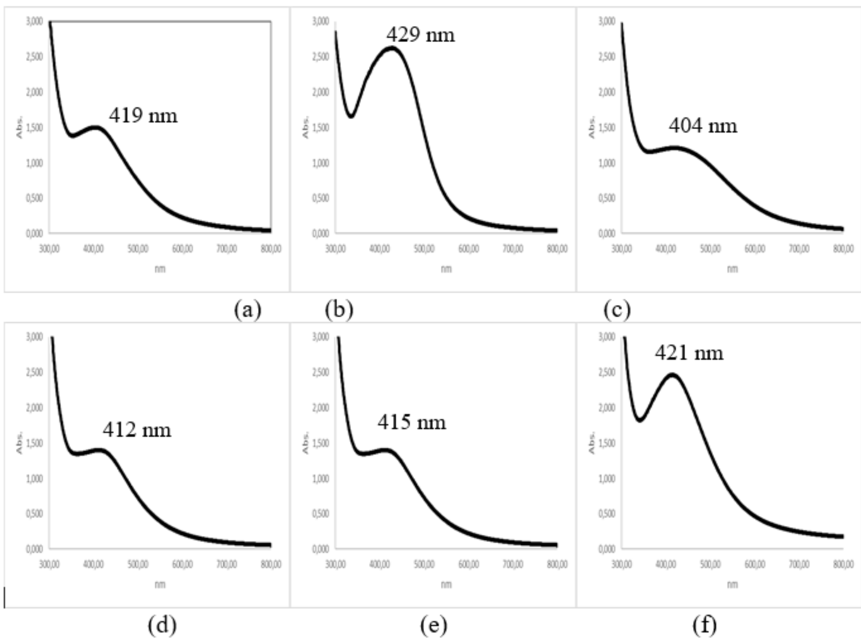

Figure 1. Screening results of endophytic bacteria from Dahlia tubers (BEUD) as bioreductors in the synthesis of silver nanoparticles (AgNPs).

The results showed that six endophytic bacterial isolates from Dahlia pinnata tubers (BEUD) exhibited different abilities to reduce silver ions (Ag^+) into silver nanoparticles (AgNPs). This variation was indicated by the absorption wavelength range of 404–429 nm, which corresponds to the characteristic surface plasmon resonance of AgNPs, as well as differences in absorbance values among the isolates [10].

Isolate BEUD2 showed the highest absorbance at a wavelength of 429 nm (2.627), followed by BEUD5 (2.470), while the other isolates exhibited lower values. These differences in bioreduction capability are associated with variations in secondary metabolites produced by each isolate, such as reductase enzymes, proteins, and phenolic compounds that play roles in the reduction and stabilization of nanoparticles [11].

IV. Conclusion

Based on the research results, six endophytic bacterial isolates from Dahlia tubers (BEUD) exhibited diversity in both macroscopic and microscopic characteristics. All isolates showed positive catalase activity, indicating their ability to adapt to oxidative stress. In the initial screening as bioreductors, all isolates were able to reduce silver ions (Ag^+) into silver nanoparticles (AgNPs), as indicated by the surface plasmon resonance (SPR) peak within the range of 400–500 nm. Among all isolates, BEUD2 demonstrated the highest bioreduction activity with a maximum wavelength of 429 nm, and was therefore selected as a potential candidate for further studies.

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