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**Research Article**

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# *Acinetobacter* sp. ME1: A Multifunctional Bacterium for Phytoremediation Utilizing Melanin Production, Heavy Metal Tolerance and Plant Growth Promotion

Soo Yeon LEE[1], Kyung-Suk CHO[1]✉

[1]*Department of Environmental Science & Engineering, Ewha Womans University, Seoul 03760, Republic of Korea*

**Abstract:** Microorganisms inhabiting soils contaminated with heavy metals produce melanin, a dark brown pigment, as a survival strategy. In this study, a melanin-producing bacterium, *Acinetobacter* sp. ME1, with heavy metal tolerance and plant-growth-promoting traits, was isolated from abandoned mine soil. Strain ME1 exhibited growth at concentrations of Zn up to 250 mg/L, Cd and Pb up to 100 mg/L, and Cr up to 50 mg/L. It had the ability to produce the plant hormone indole-3-acetic acid and siderophores along with 1-aminocyclopropane-1-carboxylic acid deaminase and protease activities. Additionally, it showed antioxidant activity, including catalase and 2,2-diphenyl-1-picryhydrazyl (DPPH) scavenging activities. The optimal conditions for melanin production by ME1 were a pH of 7 and a temperature of 35 °C. At 1000 mg/L, ME1-extracted melanin exhibited DPPH radical scavenging activity (25.04±0.007%), a sun protection factor of 15.2±0.260, and 19.6% antibacterial activity against the plant pathogen *Xanthomonas campestris*. Furthermore, its adsorption capacity was 0.235±0.073 mg/g-melanin for Zn and 0.277±0.008 mg/g-melanin for Ni. In plants of *Brassica chinensis* grown under conditions of hydroponic cultivation with single heavy metal contamination of Cd, Zn, Pb, or Cr, the removal efficiency of each heavy metal was improved by 1.1 to 2.8 times after 3 days following inoculation with the strain ME1 compared to plants grown in the same conditions without inoculation. In addition, ME1 inoculation improved the removal efficiency of each heavy metal by 1.1-2 times under multiple heavy metal contamination conditions. These findings suggest that *Acinetobacter* sp. ME1 could be used to enhance phytoremediation efficiency in heavy metal-contaminated soils. Moreover, the melanin it produces also holds promise in cosmetics, household products, and medical applications due to its photoprotective, antioxidant, and antimicrobial activities.

**Key words:** *Acinetobacter*; Melanin; Heavy metal tolerance; Plant-growth promotion; Multi-function



## 1 Introduction

Melanin is a dark brown pigment found in microorganisms, plants, and animals and is generally known to enhance the survivability of organisms (Tran-Ly et al., 2020). Metal ions, being positively or negatively charged, can easily bind to the extensive surface area of melanin (Manirethan et al., 2018), giving it the ability to chelate metal ions. Therefore, melanin can be used to detoxify or remove metal ions in heavy metal-contaminated environments while protecting cells from various biochemical hazards, including heavy metal stress (Manirethan et al., 2018; Rizvi et al., 2019). For example, *Azotobacter chroococcum*, *Klebsiella* sp. GSK, and *Pseudomonas stutzeri* have been reported to produce brown or black pigments (including melanin) that minimize heavy metal toxicity (Manirethan et al., 2018; Rizvi et al., 2019).

✉ Kyung-Suk CHO, kscho@ewha.ac.kr
Kyung-Suk CHO, https://orcid.org/0000-0002-2544-077X
Soo Yeon LEE, https://orcid.org/0000-0001-9799-4707

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Melanin has some other health benefits. For example, it can absorb a wide range of radiation, and therefore can protect cells from ultraviolet light (Rizvi et al., 2019). In the cosmetics industry, melanin is widely applied in the production of sunscreens and similar products due to its ultraviolet-blocking properties (Choi, 2021). Melanin has also been reported to protect cells from various environmental stressors, such as chemicals and high temperatures, and exhibit various biological activities, including antimicrobial, anti-inflammatory, and anticancer properties (Pandey et al., 2023; Rizvi et al., 2019). Melanin is considered environmentally friendly and biocompatible, as it is naturally synthesized in most organisms (Tran-Ly et al., 2020).

Heavy metals continuously accumulate in the soil environment, being supplied through various natural and anthropogenic sources (Hayat et al., 2023). Due to their industrial significance, heavy metals will continue to be added anthropogenically, and due to their toxicity, they will continue to pose threats to human health and have harmful effects on ecosystems (Hayat et al., 2023). Phytoremediation methods using plants and microorganisms to remove heavy metals are cost-effective and environmentally friendly solutions (Hayat et al., 2023).

Microorganisms are used to maximize the efficiency of phytoremediation. The microorganisms involved need to have mechanisms enabling flexibility in the face of environmental stressors, such as temperature, moisture content, and salinity, as well as direct resistance to heavy metal toxicity (Hayat et al., 2023). Thus, multifunctional microbial resources with heavy metal tolerance and the ability to produce melanin that are also capable of promoting plant growth could maximize the efficiency of biological restoration. However, such strategies have not yet received much attention (Rizvi et al., 2019), possibly due to insufficient research into these multifunctional microbial resources.

Therefore, in this study we aimed to isolate multifunctional bacteria from mining soil. We report the isolation of *Acinetobacter* sp. ME1, a bacterial species exhibiting heavy metal tolerance, plant growth-promoting abilities, and the capability to produce melanin. The strain's heavy metal tolerance was investigated, as were its abilities regarding several traits associated with plant growth promotion. The optimal culture conditions for melanin production by strain ME1 were determined, and the antioxidant capacity, sunscreen properties, and heavy metal removal activity of the extracted melanin were evaluated. In addition, to evaluate the applicability of phytoremediation, the effect of inoculation of *Brassica chinensis* with the ME1 strain on heavy metal removal efficiency was assessed under hydroponic cultivation conditions. Our research findings provide a better understanding of the functions of the multifunctional melanin-producing bacterium ME1 under heavy metal contamination conditions and support the use of ME1 for heavy metal removal to improve phytoremediation performance.



## 2 Materials and methods

### 2.1 Isolation and identification of melanin-producing soil bacteria

To explore bacterial resources capable of producing melanin pigments, soil samples were collected from within 2 km of a defunct mine (zinc, etc.) located in Seobuk-gu, Cheonan-si, Chungcheongnam-do (36°91'N, 127°26'E). Soil to a depth of 10 cm was collected, air-dried for about 12–14 h, sieved through a 2-mm sieve (DAIHAN®, South Korea), and stored at 4 °C until further use.

In a 20-mL test tube, 1 g of soil and 9 mL of sterilized water were mixed at 1,200 r/min for 1 min, and after standing for 15 min, the supernatant was collected. Each supernatant was then diluted stepwise up to $10^{-4}$, and 200 μL was inoculated onto modified tyrosine agar plates. The composition of the modified tyrosine agar medium was 10 g/L starch, 1 g/L L-tyrosine, 1 g/L L-asparagine, 0.5 g/L $K_2HPO_4$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L NaCl, 0.001 g/L $FeSO_4 \cdot 7H_2O$, 0.001 g/L $MnCl_2 \cdot 7H_2O$, 0.001 g/L $ZnSO_4 \cdot 7H_2O$, and 15 g/L agar at pH 7.2 (Ghadge et al., 2020). The plates were then incubated at 35 °C for 72 h and, based on the color and morphology of the colonies, a total of 8 strains (ME1–ME8) capable of producing melanin were selected, among which strain ME1 showed the most distinct melanin pigment formation (Fig. S1). Strain ME1 was then identified by analyzing a partial nucleotide sequence of the 16S rRNA gene (Lee et al., 2021a).

Colony material was suspended in 30 μL of 3rd generation distilled water, thoroughly mixed, and centrifuged at 11,000 r/min for 5 s. Then, the cells were lysed by three rounds of heating at 95 °C for 15 min using a heat block followed by centrifugation at 11,000 r/min for 5 s. The extracted genomic DNA was used as a template for polymerase chain reaction (PCR) using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), which target the 16S rRNA gene, and PCR conditions based on previous studies (Lee et al., 2021a). The amplified PCR products were sent to Macrogen Inc. for analysis. The resulting sequence was compared to those in the GenBank database using the Basic Local Alignment Search Tool (BLAST) on The National Center for Biotechnology Information (NCBI) website for identification. The partial nucleotide sequence of the 16S rRNA gene of strain ME1 was deposited in GenBank (Accession no. OR775530).

### 2.2 Evaluation of the heavy metal tolerance, plant growth promotion-associated traits, and antioxidant activity of *Acinetobacter* sp. ME1

*Acinetobacter* sp. ME1 was inoculated into 1/10 diluted LB agar medium (1 g/L tryptone, 0.5 g/L yeast extract, 1 g/L NaCl) and then incubated at 35 °C for 48 h with shaking at 120–140 r/min. The cultured broth was then centrifuged for 5 min at 5,000 × *g* to collect the bacterial cells. To wash the cells, they were resuspended in 10 mL of sterile water, and after centrifugation again to collect the cells, this washing process was repeated twice. The resulting bacterial cell suspension, adjusted to an optical density (OD) of 0.4 at 600 nm with sterile water, was used as an inoculum for evaluating heavy metal tolerance, plant growth promotion, and antioxidant activity.

The details of the methods used to investigate heavy metal tolerance, plant growth promotion associated traits, and antioxidant activity are described in the Supplementary materials and methods.

### 2.3 Optimal conditions for melanin pigment production by *Acinetobacter* sp. ME1

To investigate the optimal conditions for melanin pigment production, we prepared a culture broth by growing *Acinetobacter* sp. ME1 in modified tyrosine agar at 35 °C with agitation at 140 r/min. The culture broth obtained from this cultivation was washed with distilled water until the optical density at 400 nm reached 1 or higher, resulting in the preparation of 250 mL of inoculum.

To optimize the culture conditions to achieve high melanin pigment yields from strain ME1, we used Taguchi analysis, a factor design tool (Tarangini and Mishra, 2014). Three essential factors, pH, temperature, and inoculum amount were configured (Table S1). Using the L18 orthogonal array technique, we conducted 18 different experiments to execute the Taguchi method. The modified tyrosine agar was adjusted to pH levels of 6, 7, and 8, with 10 mL aliquots dispensed into 50-mL conical flasks for each condition. The prepared inoculum was added at 1% (v/v) and 10% (v/v) for each condition. The flasks were then incubated at temperatures of 25, 30, and 35 °C for 24 h with agitation at 140 r/min. After cultivation, the yield of melanin per unit dry cell weight (mg/g-DCW) was measured.

Based on the results obtained, we identified the optimal parameters and conducted an analysis of variance (ANOVA) to examine the significance of the results. Using the Minitab statistical program (v17.0), we determined the effects of variables individually and in combination to ascertain the optimal levels for maximizing melanin production.

### 2.4 Extraction of the melanin pigment produced by *Acinetobacter* sp. ME1

The extraction of the melanin pigment produced by strain ME1 was carried out according to the procedure shown in Fig. 3a (Ghadge et al., 2020). The culture broth of strain ME1, cultivated in modified tyrosine agar, was adjusted to an optical density of 1 or higher at 400 nm. The broth was then centrifuged at 5000 × *g* for 15 min to remove cells and debris.

The supernatant was collected, and the pH was adjusted to 2 using 6 mol/L HCl, followed by the precipitation of melanin for 72 h. Then, the solution was centrifuged at 9000 × *g* for 15 min to obtain the melanin pellet. The melanin pellet was washed 2–3 times with 1 mol/L NaOH and centrifuged at 9000 × *g* for 15 min to extract pure melanin powder. The melanin powder was dried at 70 °C for about 1 h and stored at −20 °C until further use.

To investigate the maximum absorption wavelength of the extracted melanin pigment (Shanuja et al., 2018), it was reconstituted in 1 mol/L NaOH at a concentration of 1000 mg/L. A UV–visible spectral analysis was then performed using a UV–visible spectrophotometer, measuring the spectrum in the range of 200 to 800 nm with a slit width of 1 cm in a 0.7-cm wide quartz cuvette.

## 2.5 Assessing the characteristics of the extracted melanin

### 2.5.1 Antioxidant enzyme activity (2,2-diphenyl-1-picrylhydrazyl, DPPH)

To investigate the antioxidant properties of the extract (Peng et al., 2023), the melanin powder was dissolved in 1 mol/L NaOH to prepare test samples at concentrations of 100, 250, 500, 750, and 1000 mg/L. The test samples (1 mL) were mixed with 1 mL of 0.2 mmol/L DPPH solution (in ethanol) and incubated in a dark environment at room temperature for 30 min. Absorbance was then measured at 517 nm. Ethanol at 99.9% (v/v) was used as a control. The DPPH radical scavenging activity was quantitatively evaluated using the formula (1).

### 2.5.2 Sun protection factor (SPF)

To evaluate the SPF index of the extracted melanin (Rudrappa et al., 2023), melanin powder was dissolved in 1 mol/L NaOH to prepare test samples at concentrations of 100, 250, 500, 750, and 1000 mg/L, and absorbance values in the range of 290 to 320 nm were measured for each prepared sample. The SPF index was determined using the formula

$$\mathrm{SPF} = \mathrm{CF} \times \sum_{290}^{320} EE\,\lambda \times I[\lambda] \times Abs[\lambda]$$

where CF represents the correlation factor (10), EE[λ] is the erythema effect spectrum at wavelength λ, and Abs[λ] represents the absorption at wavelength λ. The value of EEλ × I[λ] is a constant based on the wavelength (see Table S2 for specific values). The sum of the calculated values at all wavelengths represents the SPF of the sample.

### 2.5.3 Heavy metal removal efficiency and adsorption capacity

To investigate the heavy metal removal efficiency and adsorption capacity of melanin, 1000-mg/L solutions of $CuSO_4 \cdot 5H_2O$, $Pb(NO_3)_2$, $CdCl_2 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, $Cr_2K_2O_7$, and $NiCl_2$ were prepared using 5% (v/v) HNO3. Sterilized filter membranes (0.45 μm) and syringes were used for filtration and sterilization to prepare stock solutions of heavy metals. Stock solutions of Cu, Pb, Cd, Zn, Cr, and Ni were diluted to final concentrations of 20 mg/L. In addition to single heavy metal solutions, a mixed solution containing all six heavy metals at 20 mg/L each was prepared to determine the selectivity of melanin for heavy metals.

1 g of extracted melanin powder was added to 10 mL of the single and mixed heavy metal solutions. To optimize the adsorption process, the mixture was stirred at room temperature at 150 r/min for 24 h. After 24 h, the mixture was centrifuged at 12,000 r/min for 10 min, and the amount of residual heavy metals in the supernatant was analyzed using ICP-MS. The heavy metal removal efficiency (Y) and the amount of adsorbed heavy metal ions per unit mass of melanin (qt) were then calculated (Kim et al., 2012; Manirethan et al., 2018; Tran-Ly et al., 2020):

$$Y(\%) = \frac{C_0 - C_e}{C_0} \times 100$$

$$q_t\,(\mathrm{mg}\cdot\mathrm{g}^{-1}) = \frac{(C_0 - C_e)\times V}{m}$$

where C0 represents the initial concentration of metal ions (mg/L), Ce represents the concentration of metal ions remaining in the supernatant after 24 h (mg/L), V is the volume of the metal solution (L), and m is the amount of adsorbent (melanin) used in the adsorption process (g).

2.5.4 Anti-phytopathogenic activities

To investigate its antimicrobial activity against plant pathogens, we evaluated the antibacterial and antifungal properties of the extracted melanin using one strain of a plant pathogenic bacterium, *Xanthomonas campestris* KACC 10377, and three strains of plant pathogenic fungi, *Rhizoctonia solani* AG-4 KACC 40141, *Fusarium fujikuroi* KACC 46888, and *Botrytis cinerea* KACC 40573 obtained from the Korea Agricultural Culture Collection (KACC). These bacteria and fungi were cultured on potato dextrose agar (PDA medium, MBcell, USA) for 7 d. Subsequently, 10 mL of PD medium was added to each culture and shaken at 25 °C for 24 h (140 r/min). After centrifugation for 5 min at 5000 × *g*, the resulting pellets were washed with sterile water, resuspended in 10 mL of sterile water, and centrifuged again to collect the pellets. This washing process was repeated twice, and the washed pellets were resuspended in sterile water to an $OD_{600\ nm}$ of 1. The bacterial and fungal suspensions were then prepared.

The antimicrobial and antifungal activities of the selected strains were then assessed (Ghadge et al., 2020): LB and PDA agar were mixed in a 1:1 ratio (v/v) and dispensed into Petri dishes (90 mm in diameter) at 10 mL per dish. Five 6-mm paper discs were placed evenly on each petri dish, with one disc positioned at the center and the other four equidistant from each other around the center. The central disc was inoculated with 6 μL of the bacterial or fungal suspension ($OD_{600\ nm}$ = 1). The four peripheral discs were inoculated with a 1000 mg/L solution of melanin powder dissolved in 1 mol/L NaOH. Petri dishes inoculated with a commercial synthetic melanin solution (1000 mg/L; Sigma Co.) were used as positive controls, while petri dishes inoculated with 1 mol/L NaOH solution served as negative controls (untreated group). The distance between the plant pathogenic fungi and the inoculated melanin solution was measured after incubating at 35 °C for 14 d or until the fungal mycelia covered the entire dish, and the inhibition rate was calculated:

$$\text{Inhibition rate}\ (\%) = \left[\frac{(R - r_t)}{r_c}\right]\times 100$$

where *R* is the distance (mm) between the plant pathogenic fungi and the inoculated discs, $r_t$ is the growth radius (mm) in the experimental or positive control groups, and $r_c$ is the growth radius (mm) in the untreated group (negative control).

## 2.6 Experimental setup for hydroponic cultivation

To evaluate the potential for the application of phytoremediation with the ME1 strain, a hydroponic cultivation experiment was set up as illustrated in Fig. S5 (Qadir et al., 2023). For the treatment of the hydroponic solution with heavy metal contamination, solutions were prepared using $CdCl_2{\cdot}H_2O$, $ZnSO_4{\cdot}7H_2O$, $Pb(NO_3)_2$, and $Cr_2K_2O_7$ at concentrations of 30, 100, 50, and 10 mg/L, respectively, followed by sterilization using a 0.45-μm filtration filter and syringes. A singly contaminated solution containing each heavy metal added alone (Cd, Zn, Pb or Cr) and a multiply contaminated solution containing all heavy metals (Cd+Zn+Pb+Cr) were prepared. *B. chinensis*, belonging to the Brassicaceae family and used extensively in phytoremediation technology, was used in the hydroponic cultivation experiment. The cell suspension of ME1 strain was prepared as follows. The strain ME1 was cultured in a 1/10 medium for 2 days (30 °C, 120 r/min), and the culture broth was then centrifuged (13,000 r/min, 10 min) to recover the cell biomass, which was washed with sterilized distilled water. Sterilized distilled water was then added to the washed biomass to reach an OD600 nm of 0.6.

40 mL of the solution containing heavy metal was added into a 50-mL test tube. A total of 30 test tubes containing solutions of five types of heavy metals (Cd alone, Zn alone, Pb alone, Cr alone, and Cd+Zn+Pb+Cr)

were prepared, six for each type. Among the six test tubes for each type, 4 mL of the cell suspension of ME1 was inoculated into three test tubes.

Fifteen of the 50-mL centrifuge tubes received a 10% (v/v) inoculation of the prepared biomass suspension, while the remaining 15 received an equal volume of distilled water. One seedling of *B. chinensis* was planted in each of the 30 test tubes. The seedlings were cultivated under controlled light conditions of 16 h light and 8 h dark at 25 °C. After 3 days, the concentrations of heavy metals in the hydroponic solution were analyzed using ICP-MS, and the removal efficiencies of the heavy metals were calculated using the formula in section 2.5.3.

### 2.7 Statistical analysis

All experimental results were conducted in triplicate, and the mean values and standard deviations are presented. In addition, *t*-tests and one-way ANOVAs followed by Tukey's tests were conducted using R software (v4.0.1) using a *P*-value significance threshold of 0.05.

## 3 Results

### 3.1 Characteristics of *Acinetobacter* sp. ME1 isolated from abandoned mine soil

Among the eight melanin-producing strains (ME1–ME8) isolated from the abandoned mine soil samples (El-Naggar and El-Ewasy, 2017), strain ME1 was selected as it exhibited the highest melanin production and most growth (Fig. S1). Based on its partial 16s rRNA gene sequence, strain ME1 was identified as belonging to the genus *Acinetobacter*, with a similarity of 98.1%. The relationship between strain ME1 and other bacteria within *Acinetobacter* is shown in Fig. 1. Some bacteria belonging to this genus are known to have high resistance to heavy metals and improve plant growth by enhancing plant antioxidant systems (Abbas et al., 2020; Yasin et al., 2019). Additionally, it has been reported that some *Acinetobacter* species can produce melanin pigments with antioxidant activity (Coelho-Souza et al., 2013; Loi et al., 2020).

#### 3.1.1 Heavy metal tolerance

A quantitative evaluation of the effects of six heavy metals (Cd, Zn, Cu, Pb, Cr, and Ni) on the growth of strain ME1 is summarized in Fig. S2 and Table 1. Strain ME1 was able to grow significantly at concentrations of 100 mg/L or higher of Cd and 250 mg/L of Zn (Fig. S2a and b). For Cu, significant growth was observed only up to a concentration of 15 mg/L, indicating a tolerance about 17 times lower than that to Zn, the heavy metal to which ME1 was most tolerant (Fig. S2c). Growth was almost completely inhibited at concentrations above 100 mg/L for Pb (Fig. S2d), while strain ME1 exhibited tolerance at concentrations of 50 mg/L for Cr (Fig. S2e). For Ni, high growth levels were observed up to a concentration of 8 mg/L, indicating about 31 times lower tolerance compared to Zn (Fig. S2f). Therefore, strain ME1 exhibited the highest tolerance to Zn, Cd, and Pb, followed by Cr. Among the six heavy metals tested, it showed the lowest tolerance to Cu and Ni.

The lethal dose ($LD_{50}$) at which 50% of growth was inhibited due to heavy metal toxicity was highest for Cd (67.58±1.910 mg/L), followed by Pb (42.18±0.955 mg/L), and Cr (37.55±0.764 mg/L) (Table 1). The $LD_{50}$ values for Zn and Cu were similar, at 16.86±0.566 and 10.91±0.323 mg/L, respectively, while the $LD_{50}$ for Ni was the lowest at 5.57±0.159 mg/L, indicating that among the six types of heavy metals tested, Ni had the greatest impact on the growth of strain ME1. The maximum tolerable concentrations of heavy metals for growth of strain ME1 were also lowest for Ni (8 mg/L) and highest for Zn (250 mg/L) (Fig. S2).

#### 3.1.2 Plant-growth promotion and antioxidant enzyme activity

The activities of various plant growth-promoting traits in strain ME1 are summarized in Table 1. Nitrogen-fixing bacteria convert nitrogen into nitrate, supplying a usable nitrogen source for plants (Kim et al., 2020).

Strain ME1 showed nitrogen-fixing ability and an excellent capacity for producing the plant growth hormone auxin, specifically indole-3-acetic acid (IAA) (Oh et al., 2021). However, the ability to degrade the plant stress-associated molecule 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene (Mou et al., 2020), was almost negligible. The ability to solubilize phosphate and produce siderophores to assist in iron uptake within plants was high, as indicated by the formation of halo zones of 10 mm or more.

Hydrolysis is important for maintaining soil fertility and plant productivity, as externally secreted enzymes, such as proteases that hydrolyze macromolecules like proteins, can directly inhibit plant pathogenic bacteria (Lee et al., 2021b). Strain ME1 showed extracellular enzyme activity, exhibiting protease activity at a level of 1.42 μmol tyrosine/g-DCW/h. Overall, these tests suggest that strain ME1 can be presumed to contribute to maintaining plant productivity and inhibiting the growth of plant pathogens through its excellent IAA activity, nitrogen-fixing ability, phosphate solubilization, siderophore production, and protease activity.

The antioxidant enzyme activity of strain ME1 is presented in Table 1. Catalase activity was observed at a level of 37.5 units, and its DPPH radical scavenging ability was 35.7%, indicating significant antioxidant activity.

### 3.2 Optimal culture conditions for melanin pigment production by *Acinetobacter* sp. ME1

To determine the optimal conditions for melanin production by strain ME1, culture conditions were varied according to a Taguchi analysis. The amount of melanin produced under each condition is shown in Fig. 2. The maximum melanin production (129.96 mg/g-DCW) was obtained when the inoculant ($OD_{400}$ > 1) was added at 1% (v/v), the culture was at pH 7, and the incubation temperature was 35 °C. All factors were evaluated as "main effects" (Fig. S3a), and the impact of each of these three main effects on melanin production was evaluated (Table 2). The *F*-value represents the relative contribution of the estimated variance to residual variance, with a larger *F*-value indicating that the associated parameter is more important for finding the optimal conditions (Tarangini and Mishra, 2014). Furthermore, the statistical significance of the main effect can be determined through the *P*-value (Tarangini and Mishra, 2014). While pH was a significant factor in inducing optimal melanin production ($P < 0.05$), temperature and inoculum size were not significant ($P > 0.05$). The interaction and optimal levels of main effect factors were represented through response curves (Fig. S3b), indicating that maximum melanin yield could be obtained at neutral pH and temperatures above about 33.8 °C.



### 3.3 Characteristics of extracted melanin pigment

#### 3.3.1 Maximum absorption wavelength

Melanin extracted from strain ME1 exhibited a maximum absorption peak at 294 nm (Fig. 3b). The absorbance values at all concentrations showed an exponentially decreasing trend within the wavelength range of 200 to 800 nm, which is a characteristic of melanin (Ghadge et al., 2020). As mentioned in previous studies, the slope value at a concentration level of 1000 mg/L of the extracted melanin ($y = -0.0016x+1.3347$, $R^2 = 0.94$; Fig. 3b) was -0.0016. This falls within the slope range of -0.0015 to -0.0030, indicating a slope characteristic of melanin (Peng et al., 2023).

#### 3.3.2 Antioxidant enzyme activity

Evaluation of the antioxidant enzyme activity of the extracted melanin revealed significant differences in the free radical scavenging ability at different concentrations (Fig. 4a). As the concentration increased, the DPPH enzyme activity increased. At a concentration of 100 mg/L, no antioxidant activity was observed. At 250 mg/L, there was a very low activity of 1.48±0.004%, while at levels of 500 mg/L and 750 mg/L, activities of 14.43±0.018 and 17.68±0.012%, respectively, were observed. The activity reached its highest value, 25.04±0.007%, at a concentration of 1000 mg/L melanin.

### 3.3.3 SPF Value

The results of SPF analysis for the melanin extracted from ME1 at different concentrations (100, 250, 500, 750, and 1000 mg/L) are presented in Fig. 4b. The SPF values differed significantly depending on the ME1 concentration. At the lower concentrations of 100 and 250 mg/L, SPF values were measured at 1.8±0.005 and 4.1±0.003, respectively. Thus, in terms of the sun protection index, the photoprotection level was almost negligible for melanin at these concentrations. At levels of 500 and 750 mg/L, SPF values of 7.7±0.026 and 11.6±0.009, respectively, indicated low levels of photoprotection. The peak SPF value, measured at the highest concentration, 1000 mg/L, was 15.2±0.260, representing a medium protection level. Consequently, melanin extracted from strain ME1 at the concentration of 1000 mg/L has a moderate level of photoprotection, indicating reasonable effectiveness in blocking ultraviolet radiation.

### 3.3.4 Heavy metal removal efficiency and adsorption capacity

To evaluate the heavy metal removal ability of the extracted melanin, removal efficiency and adsorption capacity were assessed using artificially contaminated solutions of six single and mixed heavy metal solutions (Table 3). The removal efficiency was generally superior in the single-component heavy metal solutions compared to mixed-component solutions. In the mixed heavy metal solutions, the removal efficiencies ranged from 10 to 20%, with no significant difference observed among the types of heavy metals. Among the single-component heavy metal solutions, the melanin extract exhibited the highest removal efficiency with Zn and Ni, producing over 50% removal capacity (54.3±12.4 and 54.8±2.2%, respectively). For the other heavy metals, Cd and Cr showed removal efficiencies of 22.5±3.2 and 21.3±10.2%, respectively, while Pb and Cu showed removal efficiencies of 15.0±1.0 and 8.9±2.1%, respectively.

The adsorption capacity in single-component heavy metal solutions followed a similar trend to that of removal efficiency (Table 3). The adsorption capacity of melanin for Zn and Ni was the highest, at 0.235±0.073 and 0.277±0.008 mg/g-melanin, respectively. The adsorption capacities for Cd and Cr were similar, at 0.050±0.059 and 0.044±0.021 mg/g-melanin, respectively, while Cu showed an adsorption capacity of 0.024±0.006 mg/g-melanin. In contrast to the trend seen in removal efficiency, Pb exhibited a higher adsorption capacity, 0.073±0.055 mg/g-melanin, than Cd and Cr, which had higher removal efficiencies than Pb. In mixed heavy metal solutions, the adsorption capacity was below 0.1 mg/g-melanin for each of the six heavy metals, with an overall adsorption capacity, including all six heavy metals, at 0.292±0.019 mg/g-melanin, which was similar to the adsorption levels for Zn and Ni in the single-component solutions (Table 3).

### 3.3.5 Antimicrobial activity against plant pathogens

The antimicrobial effects of the 1000 mg/L melanin extract of strain ME1 against four plant pathogens (*R. solani* AG-4, *X. campestris*, *F. fujikuroi*, and *B. cinerea*) are shown in Fig. S4. The extracted melanin showed no antimicrobial activity against *R. solani* AG-4, *F. fujikuroi*, and *B. cinerea*, but induced a 19.6% growth reduction in *X. campestris* (Fig. S4a–d). For the positive control group (synthetic melanin), no antimicrobial activity was observed against all four plant pathogens (Fig. S4A–D).

## 3.4 Effect of inoculation of strain ME1 on heavy metal removal efficiency in hydroponic cultivation

The effects of inoculation of *B. chinensis* with strain ME1 on the removal efficiency of heavy metals in hydroponic cultivation are shown in Table 4. Inoculation with ME1 enhanced the uptake of metals, resulting in increased removal efficiencies of Cd, Zn, Pb, and Cr ($p<0.05$; Table 4). With ME1 inoculation, the removal efficiencies for Zn and Cd were 83.7±0.5% and 40.6±1.0%, respectively. Removal of both heavy metals after inoculation was better under single contamination conditions than under multiple contamination, with removal efficiencies 2 to 2.8 times higher than without inoculation. The removal efficiency for Pb after inoculation was

over 90% in both single and multiple contamination conditions (95.2±0.1% and 94.9±0.1%, respectively), which was 1.1 times higher than without inoculation. For Cr, the removal efficiency was over 50% regardless of single or multiple contamination (56.8±0.5% and 60.8±0.8%, respectively), which was 1.3 to 1.9 times higher than without inoculation.

## 4 Discussion

### 4.1 Heavy metal tolerance characteristics of the multi-functional melanin pigment producing *Acinetobacter* sp. ME1

The growth of strain ME1 was evaluated in media supplemented with various concentrations of heavy metals. Growth varied in the presence of different concentrations and different metals (Table 1). ME1 showed the most growth in Zn and Cd, as well as some degree of tolerance to Cr, Pb, Cu, and Ni (Table 1 and Fig. S2). Various studies have reported that bacteria belonging to the *Acinetobacter* genus can survive and grow in environments with high concentrations of heavy metals (Petrová et al., 2023). For instance, *Acinetobacter* sp. JJ10 isolated from soil near a Chinese refinery was able to grow in concentrations of up to 100 mg/L of Cd (Jiang et al., 2017), and *Acinetobacter* sp. JJ18 could grow in concentrations of up to 200 mg/L of Zn, which is similar to our findings (Jiang et al., 2017). In our study, *Acinetobacter* sp. ME1 showed the lowest tolerance to Ni, while a similar study reported that the growth of *Acinetobacter* sp. ADHR1 was inhibited in the presence of Hg and Ni (Montes-Robledo et al., 2024).

Bacteria tolerant to heavy metals have various mechanisms to help them survive and proliferate under heavy metal toxicity. These include heavy metal tolerance genes and the ability to produce chelating substances (such as melanin) (Rizvi et al., 2019). *Acinetobacter* sp. K1, isolated from soil in Slovakia, has heavy metal tolerance genes such as *cop*, *czc*, *chr*, and *ars* (Petrová et al., 2023). Additionally, *Acinetobacter* sp. ADHR1 was reported to have the *Chr*R gene, conferring tolerance to Cr (Montes-Robledo et al., 2024). Heavy metal-tolerant bacteria that have genes related to heavy metal transport are also considered to have high potential for enhancing phytoremediation efficiency (Karampatakis et al., 2024; Sharma et al., 2022). In previous studies, *Acinetobacter* species were found to have the Nramp family IRT1 gene for iron transport, along with ZRT1 and ZRPT2 genes, which serve as Zn transporters to increase metal transfer to plants, thereby improving phytoextraction efficiency (Sharma et al., 2022). Further studies are needed to investigate the presence of heavy metal tolerance or transporter genes in *Acinetobacter* sp. strain ME1.

The production of brown or black pigments (melanin) by some bacteria acts as another mechanism to reduce metal toxicity (Rizvi et al., 2019). Melanin has been shown to efficiently chelate metal ions, protecting cells from heavy metal stress (Rizvi et al., 2019). We speculated that the ME1 strain isolated in this study might acquire tolerance to heavy metals by producing melanin, a chelating substance, under metal stress conditions.

### 4.2 Plant-growth-promoting characteristics of *Acinetobacter* sp. ME1

Plant growth-promoting bacteria (PGPB) are promising as bioinoculants for phytoremediation (Wang et al., 2023). Various PGPB have been reported to promote plant growth through mechanisms such as nitrogen fixation, production of plant hormones (such as IAA), ACC deaminase activity, phosphate solubilization, siderophore production, or extracellular enzyme (protease) activity (Wang et al., 2023). Plants require a high nitrogen supply, and insufficient available nitrogen can severely inhibit growth (Kour et al., 2023). Auxins, such as IAA, are widely known as plant growth regulators involved in stem and root development (Banerjee and Roychoudhury, 2023). Bacteria with heavy metal tolerance that produce IAA exogenously have been observed to act as biofertilizers and efficient PGPBs (Banerjee and Roychoudhury, 2023). Some PGPBs induce ACC deaminase activity to degrade ACC, the precursor of the stress molecule ethylene, thereby inhibiting ethylene synthesis (Sun et al., 2022). They also solubilize insoluble phosphorus in the soil, form siderophore-$Fe^{3+}$ complexes to supply iron to plants, and enhance plant vigor by reducing plant diseases through mechanisms

such as the production of cell wall-degrading enzymes like proteases (Castaldi et al., 2021).

*Acinetobacter* sp. ME1 has various plant growth-promoting abilities (Table 1). Another *Acinetobacter* species isolated from a mine in Mexico was found to have nitrogen-fixing abilities (Herrera-Quiterio et al., 2020), and *Acinetobacter indicus* AB-ARC, which exhibits arsenic tolerance, has IAA production capability (Banerjee and Roychoudhury, 2023). Additionally, *Acinetobacter pittii* and *Acinetobacter* sp. CR 1.8 have been reported to exhibit protease production capability (Fitriyanti et al., 2017; Reddy et al., 2022). While most previous studies have shown that *Acinetobacter* species with heavy metal tolerance have only some of these PGP abilities, the ME1 strain in this study demonstrated abilities in all the tested metrics—nitrogen fixation capability, IAA production capability, phosphate solubilization capability, siderophore production capability, and protease activity—providing an advantage in promoting plant growth through multiple mechanisms.

### 4.3 Antioxidant enzyme activity characteristics of *Acinetobacter* sp. ME1

Antioxidants protect cells from free radicals (Tyrrell, 1998). Antioxidant enzymes are known to be involved in defending against oxidative damage to plants under environmental stress conditions (Abdelaal et al., 2021). In particular, bacteria having antioxidant enzyme activity can alleviate non-biological stress in plants and promote resistance to heavy metal stress (Wang et al., 2022). In this study, the ME1 strain, which had heavy metal tolerance and plant growth-promoting abilities, also had catalase activity (Table 1). Catalase (CAT) is the most potent type of antioxidant enzyme (Abdelaal et al., 2021). Similarly, *Acinetobacter baumannii* strains isolated from oil-contaminated soil in Changqing, China were found to have catalase activity (Zhang et al., 2021), and *Acinetobacte*r species have been reported to have the *Kat*E and *Kat*G genes, which induce catalase activity (Petrová et al., 2023). Therefore, we suspect that the ME1 strain in this study also has these genes, but further research is needed to confirm this.

The compound DPPH contains free radicals and is widely used to study antioxidant activity (Rani et al., 2023). Compounds capable of reducing DPPH through DPPH scavenging activity are considered antioxidants (Nadhe et al., 2020). Melanin's chemical structure contains neutralizers of reactive oxygen species (ROS) such as COOH, OH, and $NH_2$, which induce antioxidant activity (Zerrad et al. 2014). In this study, *Acinetobacter* sp. ME1 had DPPH radical scavenging ability (Table 1). We found no previous studies investigating DPPH radical scavenging ability in *Acinetobacter* species, but it has been observed in plants inoculated with *Acinetobacter* species (Kang et al., 2023). Despite being under salt stress, DPPH scavenging activity increased by more than 35% in soybean seedlings inoculated with *Acinetobacter* sp. YNA40 compared to an uninoculated control group (Kang et al., 2023). These levels are similar to those seen in our study, but unlike strains used in previous studies, the ME1 strain in our study also had heavy metal tolerance. An *Alcaligenes faecalis* strain isolated from metal-contaminated areas in Egypt showed a high DPPH radical scavenging activity (40–60%) (El-Alkshar et al., 2018), a superior activity compared to strain ME1 in our study. However, as the *A. faecalis* strain did not have plant growth-promoting abilities, *Acinetobacter* sp. ME1's potential value as a bacterial resource with multiple functions, including heavy metal tolerance, plant growth-promoting abilities, and antioxidant enzyme activity, is highlighted in this study.

### 4.4 Characteristics of the melanin produced by *Acinetobacter* sp. ME1

The optimal conditions for melanin production by *Acinetobacter* sp. ME1 (pH 7 and 35 °C) were found to be similar to those of previous studies (Hayat et al., 2023; Tarangini and Mishra, 2014). Specifically, for the significant factor in this study, pH (Fig. S3a and Table 2), maximum melanin production was achieved under neutral conditions at pH 7, consistent with previous studies (Tarangini and Mishra, 2014). The UV-Vis spectrum of the purified melanin produced by *Acinetobacter* sp. ME1 showed maximum absorption at 294 nm (Fig. 3b), with optical density gradually decreasing as the wavelength moved towards the visible spectrum. These are typical spectral characteristics of melanin absorption (Dadachova et al., 2008; El-Zawawy et al., 2024). While most melanin pigments studied so far have high UV-Vis absorption wavelengths within the ultraviolet range of

200–300 nm, there may be variation in their spectral characteristics depending on from which species they are purified (Mathew and G Bhat, 2022). For example, the maximum absorption wavelength of melanin was 212 nm in extracts from *Chroogomphus rutilus* (Hu et al., 2015), 250 nm in extracts from *Streptomyces glaucescens* NEAE-H (El-Naggar and El-Ewasy, 2017), and 300 nm in extracts from *Actinoalloteichus* sp. MA-32 (Hu et al., 2015).

### 4.5 DPPH radical scavenging activity and UV blocking ability of extracted melanin

Melanin has the potential to stabilize free radicals, such as ROS, and has high antioxidant and sun-blocking (SPF) factors (Eskandari and Etemadifar, 2021). The melanin pigment has unpaired electron-containing atoms that are excellent for removing free radicals and other ROS (El-Zawawy et al., 2024). Melanin acts as an antioxidant by engaging in a series of single-electron transfer reactions against free radicals, which can be used to minimize tissue damage caused by toxins (El-Zawawy et al., 2024). In this study, increases in melanin concentrations resulted in increases in DPPH scavenging activity, showing dose-dependent results (Fig. 4a). Notably, the DPPH radical scavenging ability of the melanin extracted from strain ME1 peaked at 25.04±0.007% at the highest concentration, 1000 mg/L. Our results corroborate previous research indicating that the antioxidant activity of melanin is dose-dependent (Rao and Rao, 2013; Arun et al., 2015).

However, previous studies have shown higher radical scavenging activities of melanin at 1000 mg/L. These have ranged from 56.58% to 68.91% for free radical scavenging and 87% to 96% for DPPH radical scavenging (Rao and Rao, 2013; Arun et al., 2015; El-Zawawy et al., 2024). The DPPH scavenging ability of melanin extracted from strain ME1 in this study was 0.3 to 0.4 compared to the lower scavenging activities reported in previous studies.

Melanin has been developed for commercial use as a UV blocker in cosmetics, household products, and food items (El-Zawawy et al., 2024). It has higher photostability and stronger UV absorption than previously known metabolites such as Porphyra-334, palythine, and mycosporine-like amino acids (Solano, 2020). Melanin has physiological and photoprotective properties that allow it to absorb a wide spectrum of UV–visible light, removing up to 90% of the energy absorbed from radiation such as heat (Eskandari and Etemadifar, 2021). At a concentration of 1000 mg/L, the melanin extracted from strain ME1 showed its highest SPF value, 15.2±0.260, indicating a photoprotective ability at the medium protection level (Fig. 4b; (Antony et al., 2023)). The U.S. Food and Drug Administration (FDA) recommends sunscreen with an SPF of 15 to protect against skin cancer, premature aging, and sunburns (Dutra et al., 2004). Therefore, melanin extracted from strain ME1 holds promise for use in formulations such as sunscreen, and may have fewer side effects than chemically synthesized sunscreens (Ali et al., 2017; El-Zawawy et al., 2024; Schneider and Lim, 2019).

### 4.6 Metal adsorption capacity of extracted melanin

Melanin is composed of active sites and functional groups that can bind to toxic metals, enabling subsequent detoxification (Maher et al., 2020). Potential sites and functional groups for metal binding reported for melanin include carboxyl groups, amines, hydroxyl groups, and carbonyl groups, which are known to be used in adaptive responses to protect cells from the toxicity of metal-free radicals (Kuttan et al., 2023; Maher et al., 2020). In this study, the extracted melanin showed chelating ability for six individual heavy metals (Cd, Zn, Cu, Pb, Cr, and Ni), with particularly high removal efficiencies (over 50%) and adsorption capacities (over 0.2 mg/g-melanin) for Zn and Ni (Table 3). Similar studies have reported that the absorption capacities of synthetic melanin nanoparticles were higher for Zn, Ni, and Co metal ions than for Cu and Pb (Darwish et al., 2021). Specifically, synthetic melanin exhibited over 88 mg of adsorption capacity per 1 g of melanin at a concentration of 50 mg/L Zn (Darwish et al., 2021). The absorption capacities of the melanin extracted from strain ME1 were in line with the previous research, with its lowest adsorption capacity being for Cu and the highest for Zn and Ni (Table 3). However, the overall heavy metal adsorption capacity of the melanin extracted from strain ME1 was much lower than previous estimates (Darwish et al., 2021). Notably, melanin extracted from fungi has

been reported to adsorb high concentrations of cations (Tran-Ly et al., 2020), making it difficult to compare adsorption capacities directly. However, the removal efficiency can be compared. Melanin extracted from *Armillaria cepistipes*, at 1000 mg/L, showed removal efficiencies of 95.9% for 0.6 mg/L Pb, 98.1% for 0.05 mg/L Cd, and 94.8% for 0.25 mg/L Ni and 91.0% for 0.12 mg/L Cr, respectively (Tran-Ly et al., 2020). In this study, despite setting all metal ion concentrations to 20 mg/L, which is about 30–400 times higher than those of Tran-Ly et al. (2020), melanin at the same concentration (1000 mg/L) had over 50% removal efficiency, which suggests the superior heavy metal removal ability of melanin from *Acinetobacter* sp. ME1 (Table 3).

### 4.7 Antibacterial activity of the extracted melanin against plant pathogens

Melanin can exert local toxic effects on the cell membranes of pathogens by reducing permeability to specific components necessary for bacterial survival or by forming barriers to prevent the release of waste generated during metabolism (Iqbal et al., 2012; Liu et al., 2014; Peng et al., 2023). Although studies evaluating the antibacterial abilities of melanin against human pathogens have been reported, mainly in the context of its commercial value in areas such as cosmetics or food (Correa et al., 2017), there have been fewer studies evaluating its antibacterial abilities against plant pathogens. Among four tested plant pathogens (*R. solani* AG-4, *X. campestris*, *F. fujikuroi*, and *B. cinerea*), melanin extracted from the ME1 strain showed 19.6% antibacterial activity against *X. campestris* at a melanin concentration of 1000 mg/L (Fig. S4c). In a similar study, melanin extracted from *Phoma* species bacteria showed excellent antibacterial activity against plant pathogens, with 100 mg/L melanin exhibiting the largest inhibition zones (18 mm) against *U. virens* (Surendirakumar et al., 2022). Additionally, studies have reported antibacterial activity against human pathogens, such as *E. coli*, *Klebsiella pneumoniae*, *Proteus* sp., and *Pseudomonas florescens*, rather than plant pathogens (Manivasagan et al., 2013; Arun et al., 2015). Melanin obtained from the fungus *Schizophyllum commune* exhibited antibacterial activity against those four pathogens (Arun et al., 2015), while melanin purified from *Actinoalloteichus* species bacteria exhibited antibacterial activity against *E. coli* and *B. subtilis* (Manivasagan et al., 2013). These variations in melanin antibacterial activity may arise due to the complex molecular arrangement and differences in the types of melanin (Correa et al., 2017; Michael et al., 2023; Tran-Ly et al., 2020). Although research on the antibacterial mechanism of melanin is scarce, it is generally believed to involve mainly the disruption of cell membrane integrity (Peng et al., 2023).



### 4.8 Effect of inoculating the strain ME1 on phytoremediation performance

The use of melanin-producing bacteria with heavy metal tolerance and PGP properties has garnered attention as an effective strategy for enhancing the phytoremediation of heavy metals (Tong et al., 2023; Muñoz-Torres et al., 2024). The findings of this study reveal that inoculating the melanin-producing, heavy metal-tolerant PGP bacterium, ME1, significantly elevates metal accumulation in plants within a hydroponic cultivation system, thereby enhancing heavy metal removal efficiency (Table 4). This is likely attributed to the strain's ability to grow at levels of Cd, Zn, Pb, and Cr, reaching up to 50–250 mg/L (Table 1). Notably, of the four types of heavy metals tested, ME1 played a significant role in removing Pb (Table 4). These results suggest that *B. chinensis* is a viable candidate for phytoremediation strategies targeting Pb (Akram et al., 2021; Canal et al., 2023), but *Acinetobacter* species are known to use mainly biological adsorption mechanisms for Pb remediation (Bazzi et al., 2020; Van Veenhuyzen et al., 2021).

In a study similar to ours, the inoculation of water hyacinth (*Eichhornia crassipes*) with *Acinetobacter lwoffii* ENSG302, which has hydroxyl, carboxyl, and phosphate groups capable of binding Pb to cell surfaces, resulted in an 89.3% Pb removal efficiency under conditions of 100 mg/L Pb contamination (Haque et al., 2021). Furthermore, under single contamination conditions for Cd, an impressive removal efficiency of 83.7% was recorded. In similar research, the inoculation of *Acinetobacter* sp. CS9 as a PGPB improved the growth of periwinkle (*Catharanthus longifolius*) while achieving over 10% Cd removal efficiency in soil contaminated with 200 mg/kg of Cd (Yasin et al., 2019). As mentioned above, the melanin production of ME1 is considered a

key mechanism for heavy metal adsorption.

These findings indicate that inoculation of *Acinetobacter* sp. ME1 improves the heavy metal removal efficiency of plants within hydroponic cultivation systems. Bacteria, like the strain ME1, that produce melanin show a high affinity for metal ions due to the various functional groups (carbonyl, hydroxyl, amino, and carboxyl groups) present in melanin, making them efficient in removing metal ions (Darwish et al., 2021). In this preliminary study we explored the potential influence of melanin-producing bacteria for phytoremediation, highlighting the imperative for further applied studies to assess the impact of multifunctional melanin-producing bacteria, such as strain ME1, on phytoremediation within heavy metal-contaminated field soils.

## 5 Conclusions

There is a need to explore the potential for using multifunctional heavy metal-resistant bacteria as a new strategy for the phytoremediation of heavy metal-contaminated soils. This study aimed to investigate the characteristics of multifunctional bacterial resources by isolating them in their pure form and demonstrating their potential for applications in environmental restoration, agriculture, cosmetics, and medical fields. We specifically focused on their melanin pigment extracts. *Acinetobacter* sp. ME1 was isolated from a mining area. It exhibited not only excellent heavy metal resistance but also plant growth-promoting and antimicrobial properties against plant pathogens. It had a notable ability to produce melanin pigments, which play a role in heavy metal defense as chelators. Melanin produced by the multifunctional bacterial strain ME1 exhibited UV-blocking ability, antioxidant enzyme activity, adsorption capability for various types of heavy metals, and antimicrobial activity against plant pathogens. Furthermore, through hydroponic cultivation experiments, we confirmed that the removal efficiency of heavy metals by phytoremediation could be improved by inoculating plants with the strain ME1 to induce heavy metal accumulation. However, additional research is required to investigate the genes associated with the heavy metal resistance and antioxidant activity of the *Acinetobacter* sp. ME1 strain, and to evaluate its potential as a biosorbent in environments contaminated with heavy metals. The multifunctional capabilities of *Acinetobacter* sp. ME1 and its melanin extract demonstrated in this study are expected to not only enhance the efficiency of soil remediation using plants and microorganisms but also find applications in industries such as cosmetics, household goods, and healthcare. Future work will focus on physicochemical characterization of the melanin extracted from strain ME1 and validating its phytoremediation potential through field trials assessing remediation efficiency in heavy metal contaminated soils using *Acinetobacter* sp. ME1.



### Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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### Author contributions

Soo Yeon LEE performed conceptualization, data curation, investigation, methodology, visualization, wrote and edited the manuscript. Kyung-Suk CHO performed funding acquisition, supervision, wrote and edited the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

### Compliance with ethics guidelines

Soo Yeon LEE and Kyung-Suk CHO declare that there are no conflicts of interest in the study.

This research does not contain any studies with human or animal subjects performed by any of the authors.

**Table 1 Heavy metal (HM) tolerance, plant growth promotion-associated traits, and antioxidant activity measures for *Acinetobacter* sp. ME1.**

| **Characteristics** | **Activity** | |
|---|---|---|
| ***Heavy metal tolerance*** | **LD50[a] (mg/L)** | **Max[f] (mg/L)** |
| Cd | 67.58±1.910 | 100 |
| Zn | 16.86±0.566 | 250 |
| Cu | 10.91±0.323 | 15 |
| Pb | 42.18±0.955 | 100 |
| Cr | 37.55±0.764 | 50 |
| Ni | 5.57±0.159 | 8 |
| ***Plant growth promoting trait*** | | |
| $N_2$ fixation ($OD_{630\ nm}$) | | 0.180±0.022 |
| IAA synthesis ($OD_{535\ nm}$)[b] | | 1.857±0.001 |
| ACC deaminase ($OD_{600\ nm}$)[c] | | 0.002±0.008 |
| Phosphate solubilization | | ++[g] |
| Siderophore production | | ++ |
| Protease activity (μmol tyrosine/g-DCW/h) | | 1.42±0.02 |
| ***Antioxidant activity*** | | |
| CAT activity (units)[d] | | 37.5±12.5 |
| DPPH radical scavenging activity (%)[e] | | 35.7±0.02 |

[a] $LD_{50}$: Heavy metal concentration decreased $OD_{600}$ value by 50%
[b] IAA: indole-3-acetic acid
[c] ACC: 1-aminocyclopropane-1-carboxylic acid
[d] CAT: catalase
[e] DPPH: 2,2-Diphenyl-1-picrylhydrazyl
[f] Max: maximum concentration possible for growth
[g] ++: positive (> 10 mm clear zone diameter)

**Table 2 Analysis of variance table for the main effect factors influencing melanin production by strain ME1.**

| **Analysis of Variance for Means** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Source** | **Df[a]** | **Seq SS[b]** | **Adj SS[c]** | **Adj MS[d]** | **F[e]** | **P[f]** | **Contribution (%)** |
| Inoculation (%) | 1 | 2020 | 2020 | 2020.2 | 4.33 | 0.059 | 12.67 |
| pH | 2 | 6095 | 6095 | 3047.7 | 6.54 | 0.012 | 38.23 |
| Temperature (°C) | 2 | 2234 | 2234 | 1116.8 | 2.4 | 0.133 | 14.01 |
| Residual Error | 12 | 5595 | 5595 | 466.2 | | | 35.09 |
| Total | 17 | 15944 | | | | | |

[a] Df: Degree of freedom

[b] Seq SS: Sequential sums of squares

[c] Adj SS: Adjusted sums of squares

[d] Adj MS: Adjusted mean squares

[e] F: F-value

[f] P: *P*-value

**Table 3 Metal removal efficiency and metal adsorption capacity of the melanin biosynthesized by *Acinetobacter* sp. ME1.**

| Metal | | Removal efficiency (%) | Adsorption capacity ($q_t$, mg/g-melanin) |
|---|---|---|---|
| In single ion solution | **Cd** | 22.5±3.2[ab] | 0.050±0.059 |
| | **Zn** | 54.3±12.4[a] | 0.235±0.073 |
| | **Cu** | 8.9±2.1[b] | 0.024±0.006 |
| | **Pb** | 15.0±1.0[b] | 0.073±0.055 |
| | **Cr** | 21.3±10.2[ab] | 0.044±0.021 |
| | **Ni** | 54.8±2.2[a] | 0.277±0.008 |
| In multi-ion solution | **Cd** | 15.1±1.7[*] | 0.030±0.004 |
| | **Zn** | 14.2±2.4[*] | 0.054±0.010 |
| | **Cu** | 14.6±0.6[*] | 0.041±0.002 |
| | **Pb** | 15.6±0.0 | 0.061±0.000 |
| | **Cr** | 13.1±0.7[*] | 0.025±0.001 |
| | **Ni** | 16.0±2.4[*] | 0.081±0.013 |
| | | **Total** | 0.292±0.019 |

Data are expressed as mean ± standard deviation, n=3. [a] Letters represent significance groupings when comparing metal removal efficiencies between types of heavy metals in single metal solutions; means with the same letter are not significantly different.

[*] Asterisks represent significant differences in metal removal efficiency between single ion and multi-ion solutions for each type of heavy metal

**Table 4 Removal efficiency (%) of Cr, Zn, Cd and Pb by *Acinetobacter* sp. ME1 in the hydroponics cultivation.**

| Heavy metal | | Treatments | |
|---|---|---|---|
| | | w/ ME1[a] | w/o ME1 |
| In single ion solution | Cd | 83.7±0.5[a*] | 78.9±0.3[b] |
| | Zn | 40.6±1.0[a] | 14.6±1.0[b] |
| | Pb | 95.2±0.1[a] | 85.0±0.2[b] |
| | Cr | 56.8±0.5[a] | 45.3±0.2[b] |
| In multi-ion solution | Cd | 32.2±2.4[a] | 21.4±1.6[b] |
| | Zn | 28.5±0.3[a] | 14.3±1.4[b] |
| | Pb | 94.9±0.1[a] | 88.6±0.2[b] |
| | Cr | 60.8±0.8[a] | 32.3±1.2[b] |

Data are expressed as mean ± standard deviation, n=3. a w/ ME1; Inoculation *Acinetobacter* sp. ME1 in the hydroponics cultivation, w/o ME1; Inoculation distilled water in the hydroponics cultivation

* Evaluation of significant difference in heavy metal removal efficiency

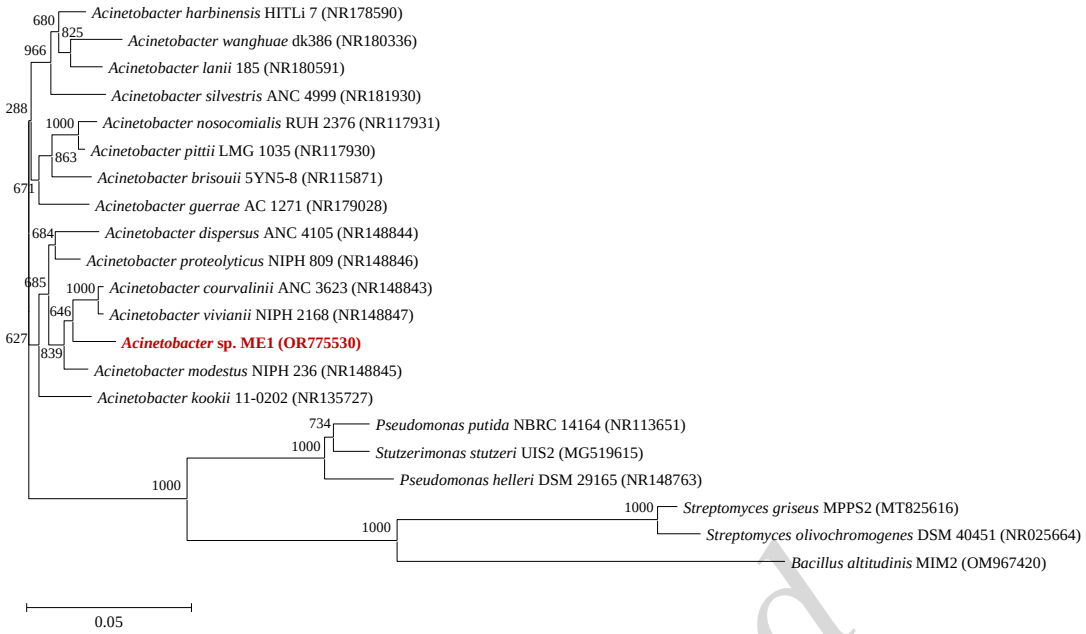


**Fig. 1**

Neighbor-joining phylogenetic tree of strain ME1, related *Acinetobacter* strains, and outgroup species estimated from 16S rRNA sequences. Bootstrap values (percentages of 1,000 resamples) are shown at the branch points. The scale bar represents 0.05 substitutions per site. The 16S rRNA gene sequences of melanin producing bacteria and bacteria similar to ME1 were used as the outgroups (Bayram, 2021; Deepthi et al., 2021; Eskandari and Etemadifar, 2020; Mira Gordhanbhai et al., 2022).

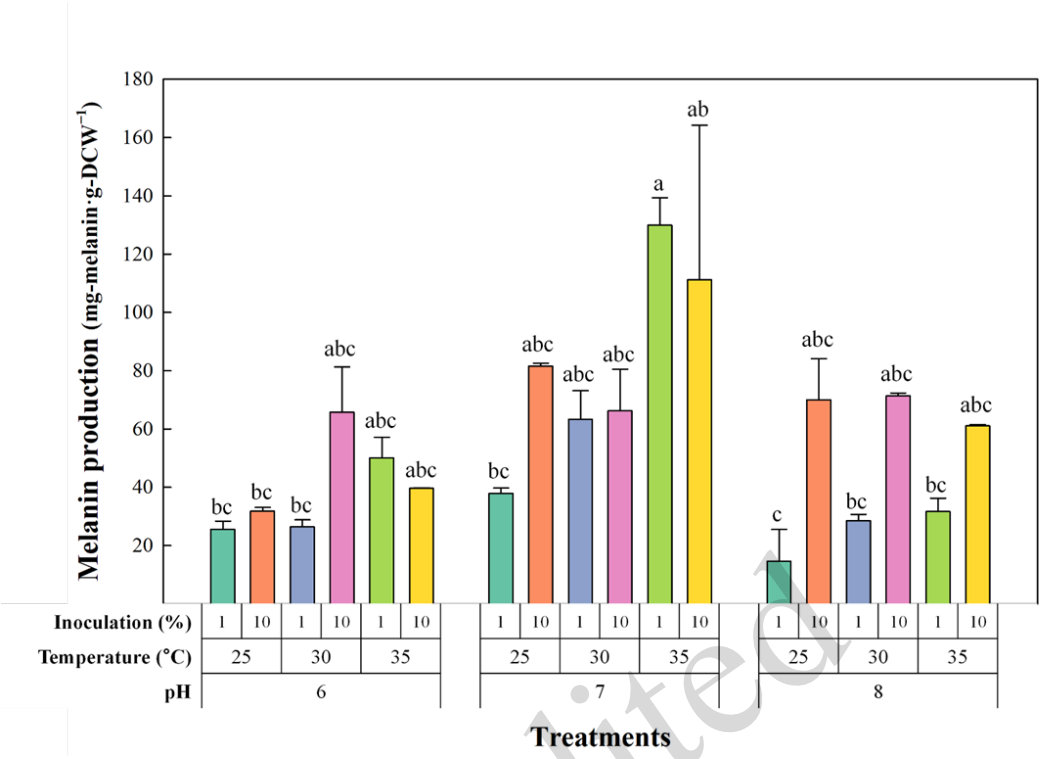


**Fig. 2**

Optimizing melanin production (mg-melanin/g-DCW) by strain ME1. Three factors, inoculation amount (%), temperature (°C), and pH, were varied and applied in 18 trials. Data are expressed as mean ± standard deviation, n=3.

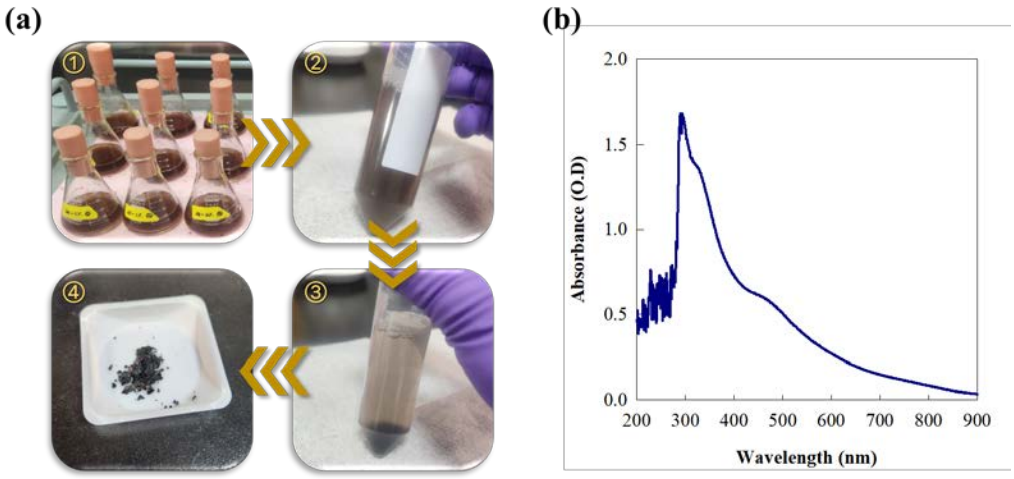


**Fig. 3**

(a) Images detailing the melanin extraction and purification procedure for strain ME1 in tyrosine broth. ① Melanin production in a liquid medium supplemented with tyrosine. ② The melanin produced was extracted by acid precipitation. ③ Precipitated melanin was washed in 1 mol/L NaOH and pelleted. ④ A close-up view of the final melanin particles biosynthesized by ME1. (b) Absorbance of the melanin extracted from ME1 in the UV–visible spectrum.

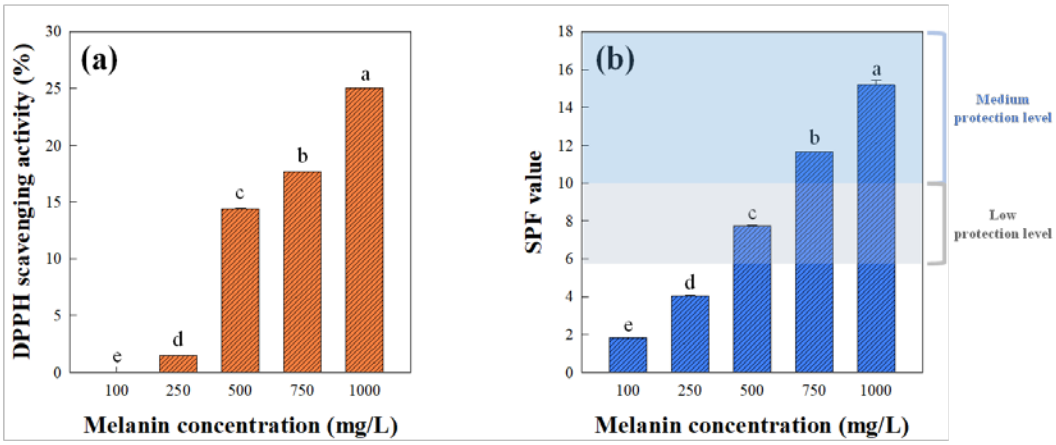


**Fig. 4**

(a) The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the ME1-extracted melanin. (b) The sun protection factor (SPF) of the extracted melanin classified by protection level (Antony et al., 2023). Lowercase letters above the bars represent significance groupings; means with the same letter are not significantly different ($P < 0.05$).

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**Supplementary information:**

Table S1-S2; Figs. S1-S5; Materials and methods