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Exploration of cadmium-resistant bacteria in cadmium-contaminated rice fields in Karawang District, Indonesia

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Abstract. *Azis NR, Nuraini Y, Prayogo C, Ishaq RM, Nuttapol N. 2024. Exploration of cadmium-resistant bacteria in cadmium-contaminated rice fields in Karawang District, Indonesia. Biodiversitas 25: 3637-3644.* Cadmium is a heavy metal naturally present in the soil, but its presence can increase due to human activities (anthropogenic). Increased cadmium content in soil due to human activities can occur due to agricultural activities, such as the continuous use of chemical fertilizers and pesticides. These chemical products contain harmful heavy metals such as cadmium, which results in soil pollution due to the accumulation of these metals in the soil and the entry of these metals into agricultural products, thus affecting health. The problem can be solved by soil bioremediation that utilizes indigenous bacteria. This study aims to explore cadmium-resistant bacteria with high resistance levels that can be used for the bioremediation of cadmium-contaminated soil. Cadmium-resistant bacteria were explored from 4 locations in Karawang District, Indonesia, with soil cadmium content >0.5 ppm, namely 2 locations in Telukjambe Village, 1 in Purwajaya Village, and 1 in Lemahmukti. 14 cadmium-resistant bacterial isolates with different characteristics were obtained from these 4 locations. Microbiological tests conducted include Gram staining test, resistance test, pathogenicity test, and bacterial DNA test. Seven bacterial isolates were found to be non-pathogenic to humans and plants. The best three bacterial isolates from 7 non-pathogenic isolates with resistance ability 50-500 ppm can potentially be used as a bioremediation of cadmium-contaminated soil. The three isolates are isolates B1, B6, and B12. DNA test results stated that isolate B1 is *Shewanella decolorationis* strain NSSD01, B6 is *Chryseobacterium cucumeris* strain E1-3, and B12 is *Aeromonas hydrophila* strain GX5.

Keywords: Bioremediation, cadmium, cadmium-resistant bacteria, exploration, soil pollution

INTRODUCTION

Cadmium (Cd) is a heavy metal naturally occurring in soil, water, and groundwater at low concentrations. Cadmium content in soil ranged from 0.01-1 mg/kg with an average of 0.36 mg/kg, while soil, water, and groundwater ranged from 1-5 μg/L (Kubier et al. 2019). Cadmium naturally comes from the weathering of rocks containing Cd, such as basalt rocks (0.006-0.6 ppm Cd), granite (0.003-0.18 ppm Cd), and sedimentary rocks (<0.3-8.4 ppm Cd) (Alengebawy et al. 2021). Cadmium in the environment can increase as a result of human activities (anthropogenic), such as agricultural practices, industrial processes (metal plating, paint coloring, battery stones, waste), fossil fuels, and mining (Alengebawy et al. 2021). Agricultural activities that contribute to the input of heavy metal Cd into the soil are the use of agrochemical products in the form of fertilizers and pesticides.

The use of chemical fertilizers and pesticides can increase Cd content in the soil. The Cd content in fertilizers varies, with the average Cd content being 0.51 mg/kg with the maximum content reaching 2.13 mg/kg (Kubier et al. 2019). The contribution of phosphate fertilizers as Cd contamination due to anthropogenic activities reaches 56% (Li et al. 2021). Apart from fertilizers, agricultural chemicals such as pesticides also contain heavy metal cadmium, such as in some insecticides (Dursban, Emacel, Arjuna, Tripas, Buldok, and Marshal) and fungicides (Antracol, Folicur, Dithane, Octave) ranging from 0.04-0.50 mg/kg (Dewi et al. 2022).

The use of fertilizers and pesticides in agricultural activities results in the accumulation of Cd in the soil, which pollutes the soil and is harmful to human health when cultivated plants absorb the metal. Bioaccumulation and biomagnification of heavy metals and pesticides, especially in the food chain, cause several health problems in humans, such as fatigue, neurotoxicity, lung problems, kidney disease, skin infections, cancer growth in the human body, and can even cause death (Sarker et al. 2021; Rajendran et al. 2022).

The content of cadmium metal in agricultural products makes agricultural products the largest contributor to human exposure to cadmium metal. This is due to the high mobility of cadmium in soil, which plants easily absorb. Cadmium is absorbed through plant roots into leaves, fruits, and edible grains. Cadmium can also accumulate in animal milk and fatty tissue, leading to human exposure when consuming cadmium-containing plants or animals

(Onokebhagbe et al. 2019). The levels of Cd in agricultural products that exceed the maximum threshold of the National Standardization Agency (0.2 mg/kg) have been found, such as in spinach and kale in Tanggerang, Indonesia, which were 0.23 mg/kg and 0.3 mg/kg (Rinawati and Sofiatun 2018), in mustard greens and carrots in Denpasar, Indonesia were 1.05 mg/kg and 0.93 mg/kg (Azizah and Maslahat 2021), in rice in Hunan, China ranged from 0.042-1.415 (mg/kg) and in wheat in Zhejiang, China ranged from 0.023-1.30 (mg/kg) (Gao et al. 2021).

The problem of Cd pollution in soil can be solved by soil bioremediation. It is the process of overhauling or transforming hazardous contaminants into non-toxic compounds carried out by living organisms without any other harmful effects on the environment. The use of living organisms in the bioremediation process, especially microorganisms, is because microorganisms can produce enzymes (hydrolase, catalase, laccase, dehalo genase, etc.) that play a role in the degradation, stabilization, mineralization, sequestration, and detoxification of organic pollutants and heavy metals (Zhang et al. 2020). Some living organisms that have demonstrated their ability to remediate soil contaminated with heavy metals and pesticides are Actinomycetes, fungi, bacteria (*Pseudomonas, Bacillus, Actinobacter, Acinetobacter, Burkholderia*, and *Klebsiella*) (Sarker et al. 2021; Mgbodile et al. 2022). The Cd bioremediation can be done by utilizing indigenous bacteria that come from Cd-contaminated sites and can grow well under high cadmium stress conditions. Indigenous bacteria were chosen because they have good adaptability to Cd stress conditions, so their resistance ability can be utilized in bioremediation. Therefore, the purpose of this study is to explore non-pathogenic cadmium-resistant bacteria with high levels of resistance to cadmium stress from cadmium-contaminated agricultural land that has the potential to be used as bioremediators in cadmium-contaminated soil bioremediation efforts.

MATERIALS AND METHODS

This research was conducted in August-December 2023. The soil samples were collected from eight locations spread across Karawang District, West Java, Indonesia. The Cadmium-resistant bacteria were analyzed at the Soil Biology Laboratory, Faculty of Agriculture, Universitas Brawijaya.

Analysis of initial soil cadmium content

Three locations suspected of having high Cd content in Karawang District were selected as three locations in Darawolong Village, Purwasari Subdistrict, three locations in Telukjambe Village, East Telukjambe Sub-district, one location in each Purwajaya Village, Tempuran Sub-district and in Lemahmukti Village, Lemahabang Sub-district. These locations are rice fields with intensive use of chemical fertilizers and pesticides that lasted more than 50 years. The location in Telukjambe Village is an intensive agricultural land in addition to a textile factory whose waste disposal is not entirely per applicable regulations so there might be textile waste contamination in irrigation streams. The analysis of cadmium was conducted using the wet ignition method and the reading was done using an AAS (Atomic Absorption Spectrophotometer) at a wavelength of 228.8 nm (Eviati et al. 2023).

Characterization of cadmium-resistant bacteria

Characterization of bacteria is done by observing the morphological appearance of bacteria in terms of shape, elevation, size, color, and surface. Bacterial characterization is done using Bergey's Manual of Determinative Bacteriology (Holt et al. 1994)

Bacterial purification

Bacterial purification was done to find the best single bacterium that is resistant to Cd contamination and has the potential as a Cd bioremediator. Bacterial purification was carried out using the strake plate method. Bacterial colonies with different properties (color, size, shape) from the initial isolation results were taken using an ose needle and then scratched on NA+$Cd(NO_3)_2$ media in a petri dish, then incubated for two days. Purification was repeated 2-3 times until a pure culture was obtained (Mutaqin et al. 2017).

Gram stain test

Bacterial staining was done by adding basic dyes (crystal violet, iodine, safranin). Gram staining of bacteria facilitates the observation of bacteria under a microscope, clarifies the size and shape of bacteria to see the outer and inner structure of bacteria, and increases the contrast between microorganisms and their surroundings. Gram-positive bacteria are marked with violet color, while Gram-negative bacteria are marked with red or pink when observed under a microscope because the bacteria were unable to bind the crystal violet color (Tasripin et al. 2022).

Resistance test

A resistance test was conducted to determine bacterial isolates' resistance level to Cd stress at different concentrations. The concentration of Cd used, namely 0 ppm, 10 ppm, 20 ppm, 50 ppm, 100 ppm, 200 ppm, and 500 ppm. Bacterial isolates that can growth on media (NA+Cd $(NO_3)_2$) indicate the isolate was resistant to cadmium contamination (Fahruddin et al. 2020).

Pathogenicity test

The pathogenicity test consists of a hemolysis test and a hypersensitivity test. The hemolysis test was carried out on blood agar media to determine whether the bacteria were pathogenic to humans and animals. In contrast, the hypersensitivity test was carried out on tobacco leaves to determine whether the bacteria were pathogenic to plants. Pathogenic bacterial isolates are characterized by lysis on blood agar media and necrosis on tobacco leaves that were injected with these bacterial isolates (Bafandeh et al. 2019).

DNA testing of bacterial isolates

A bacterial DNA test was carried out using the polymerase chain reaction (PCR) method to determine the best bacteria species found. NA extraction was performed using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) (B/7.2.1/IKP/002). DNA amplification was performed using the MyTaq HS Red Mix, 2X kit (Bioline, BIO-25048) (B/7.2.1/IKP/002). Electrophoresis of 16S gene amplification products (B/7.2.1/IKP/002) Bidirectional sequencing using the Sanger DNA Sequencing by using Capillary Electrophoresis method (1st BASE Subcontract Lab Testing), and Bioinformatics analysis of Sanger Sequencing results (B/7.2.1/IKP/002).

RESULTS AND DISCUSSION

Soil cadmium content in various rice fields

The results of cadmium content analysis at the suspected sites in several intensive rice fields in Karawang District are presented in Table 1.

The results of the analysis of cadmium (Cd) content in soil of several agricultural lands in Karawang District showed that six locations were detected to contain Cd. In comparison, two other locations were not detected by the AAS (Atomic Absorption Spectrophotometer) due to very low Cd content. The Cd content in the detected locations ranged between 0.41-1.22 ppm. Not all detected sites have Cd content exceeding the threshold allowed in soil, according to the Ministry of State for Population and Environment of Indonesia, and Dalhousie, University Canada (1992), which is 0.5 ppm. Locations with Cd content >0.5 ppm are 4, 6, 7, and 8, with Cd content ranging between 0.96-1.22 ppm.

Microbiology test of cadmium-resistant bacteria

Cadmium-resistant bacteria were isolated from four sites with Cd content exceeding the threshold (>0.5 ppm), namely sites 4, 6, 7, and 8. Microbiological tests consisted of bacterial colony characterization and bacterial Gram staining tests. Characterization of bacterial colonies was carried out as a macroscopic examination assessed by differences in bacterial morphology in the form of the overall shape and shape of the colony edges, elevation, and color of the bacterial colonies. The microbiological test results of cadmium-resistant bacterial isolates are presented in Table 2.

Table 1. Soil cadmium content in various rice fields in Karawang District, Indonesia

Location	Land use	Soil C (ppm)
Darawolong Village, Purwasari Sub-district	Irrigated rice fields	0
Darawolong Village, Purwasari Sub-district	Irrigated rice fields	0,41
Telukjabe Village, Telukjame Timur Sub-district	Moorland with cucumber commodity	0,41
Telukjabe Village, Telukjame Timur Sub-district	Rice fields next to the polluted cotton mill waste	1,22*
Darawolong Village, Purwasari Sub-district	Moorland with cabbage commodity	0
Telukjabe Village, Telukjame Timur Sub-district	Rice fields next to a textile factory polluted by textile and cotton mill waste	0,98*
Purwajaya Village, Tempuran Sub-district	Irrigated rice fields	0.98*
Lemahmukti Village, Lemahabang Sub-district	Irrigated rice fields	0.96*

Note: *Locations with Cd above the standard threshold (0.5 ppm)

Table 2. Microbiological test results of cadmium-resistant bacteria

Code	Morphological characterization of bacterial colonies				Gram strain test	
	Shape	Margin	Elevation	Color	Shape	Gram
B1	Circular	Entire	Flat	Cream	Coccus	Negative
B2	Circular	Entire	Flat	Yellow-white	Bacil	Positive
B3	Circular	Entire	Flat	Cream	Coccus	Positive
B4	Circular	Entire	Flat	White	Basil	Positive
B5	Circular	Entire	Flat	White-blue	Bacil	Positive
B6	Circular	Entire	Convex	Yellow	Coccus	Negative
B7	Circular	Entire	Convex	White	Bacil	Positive
B8	Circular	Lobare	Flat	White	Bacil	Positive
B9	Circular	Entire	Convex	Reddish-white	Coccus	Positive
B10	Irregular	Undulate	Flat	White	Coccus	Positive
B11	Circular	Entire	Flat	Red	Coccus	Negative
B12	Irregular	Undulate	Flat	Yellow	Coccus	Positive
B13	Circular	Erose	Flat	White	Coccus	Positive
B14	Irregular	Undulate	Convex	Yellow	Coccus	Negative

Characterization of bacterial colony morphology was considered as the initial approach to bacterial identification. The isolation and morphological characterization of cadmium-resistant bacteria showed that there were 14 isolates with different morphologies. The shape of the bacterial colonies was found to be dominated by round, thorough edges, and irregularly shaped colonies with talc and orese edges. The elevation of bacterial colonies is dominated by flat and convex with white and yellow colors. Colony color appears due to the properties of some media or due to pigment production by bacteria. Some bacteria can produce colored colonies due to changes in pH or enzymatic activity (Fahruddin et al. 2019). Cd-resistant bacterial isolates are dominated by Gram-positive with cocci and bacilli. Gram-positive bacteria are composed of peptidoglycan, which is higher than Gram-negative bacteria, so they have a greater capacity to bind metal ions. Cell wall components that play a role in cadmium binding are exopolysaccharides (EPSs) (Xia et al. 2021).

Resistance test of cadmium-resistant bacteria

The resistance test was conducted to analyze the capacity of resistance properties of bacterial isolates found to cadmium stress with the treatment of several cadmium concentration variations, namely 0 ppm, 10 ppm, 20 ppm, 50 ppm, 100 ppm, 200 ppm, and 500 ppm. The resistance test results of cadmium-resistant bacteria are presented in Table 3.

Resistance test results showed that all bacterial isolates can grow well up to a Cd concentration of 10 ppm and begin to inhibit growth at a concentration of 20 ppm. Bacteria with a high level of resistance (>50 ppm) are B6, B12 and B14 (Figure1). The three bacterial isolates can be developed as Cd metal bioremediators if they are not included in pathogenic bacteria. Cd-resistant bacteria that can live in high Cd contamination conditions indicate that these bacteria have a high tolerance to Cd stress. Tolerance describes an individual cell response to disturbance, which affects the stability of the total community and is related to the activation of protective or adaptation mechanisms for survival. Tolerance can also be used to explain changes in the ecosystem that initially impact microbial growth to no longer impact microbial growth (Bravo and Braissant 2022).

Table 3. Resistance test of cadmium-resistant bacteria

Code	**Cd concentration**						
	0 ppm	**10 ppm**	**20 ppm**	**50 ppm**	**100 ppm**	**200 ppm**	**500 ppm**
B1	+++	+++	+++	-	-	-	-
B2	+++	+++	+	-	-	-	-
B3	+++	+++	-	-	-	-	-
B4	+++	+++	++	+	-	-	-
B5	+++	+++	+	+	-	-	-
B6	+++	+++	+++	+++	+++	++	+*
B7	+++	+++	-	-	-	-	-
B8	+++	+++	+++	+++	+	-	-
B9	+++	+++	+++	-	-	-	-
B10	+++	+++	+++	+++	-	-	-
B11	+++	+++	+	-	-	-	-
B12	+++	+++	+++	+++	+++	+++	++*
B13	+++	+++	+++	++	-	-	-
B14	+++	+++	+++	+++	+++	+++	++*

Note: (+++): excellent growth; (++): good growth; (+): poor growth; (-): no growth; *: bacteria with high resistance level

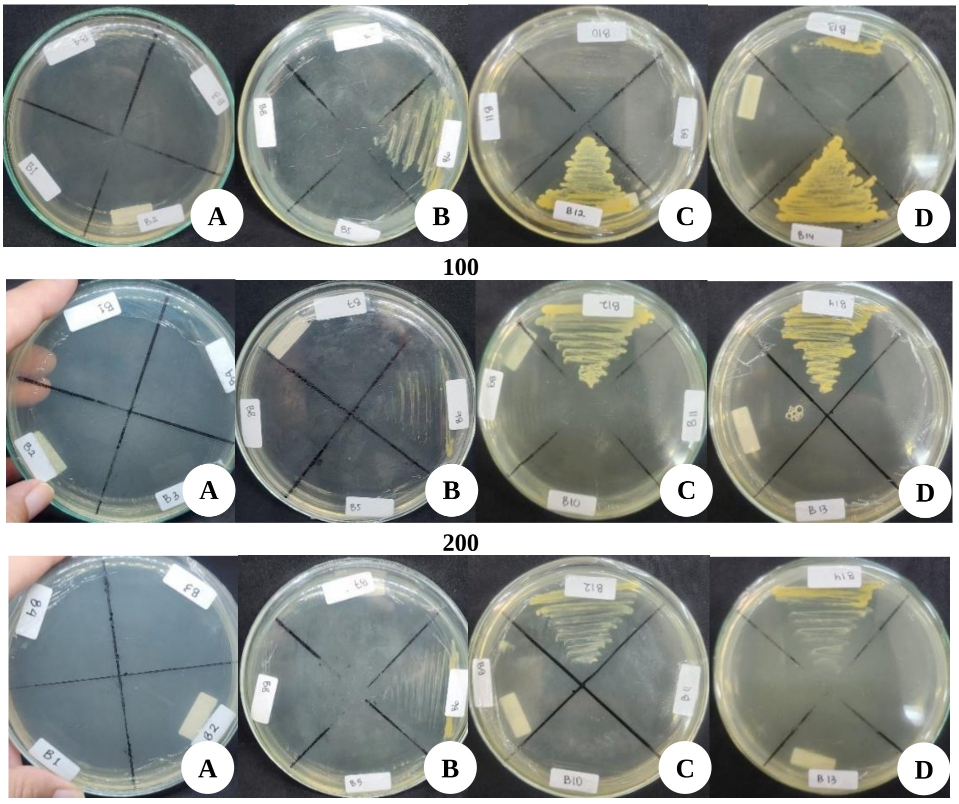

Figure 1. The resistance test results of cadmium-resistant bacteria. A. Isolate B1; B. Isolate B6; C. Isolate B12; D. Isolate B14

Pathogenicity test of cadmium-resistant bacteria

The Hemolysis and Hypersensitivity Tests were used as pathogenicity tests to review the safety of obtained Cd-resistant bacterial isolates from unexpected pathogenic potential in humans and plants. This test can eliminate a large number of harmful pathogen-positive isolates from a number of obtained Cd-resistant bacterial isolates (Amaria et al. 2023). The pathogenicity test results are presented in Table 4.

The results of the hemolysis test showed that seven isolates out of 14 bacterial isolates found were pathogenic to humans, namely isolates B4, B5, B8, B9, B10, B13, and B14. In contrast, the results of the hypersensitivity test showed that only isolate B4 was pathogenic to plants. The seven pathogenic bacterial isolates cannot be developed as bioremediation, even though they have high resistance to Cd stress. Pathogenic bacteria are characterized by lysis on blood agar media and necrosis on tobacco leaves, which are used as a medium for testing the pathogenicity of these bacterial isolates (Figure 2). Bacteria that can be utilized as bioremediators of cadmium-contaminated soil are isolates B1, B2, B3, B6, B7, B11, and B12 because they are not pathogenic to humans and plants based on the pathogenicity test results.

Table 4. Pathogenicity test of cadmium-resistant bacteria

Kode	Hemolysis test	Hypersensitivity test	Classification
B1	-	-	Non-pathogen
B2	-	-	Non-pathogen
B3	-	-	Non-pathogen
B4	+	+	Pathogen
B5	+	-	Pathogen
B6	-	-	Non-pathogen
B7	-	-	Non-pathogen
B8	+	-	Pathogen
B9	+	-	Pathogen
B10	+	-	Pathogen
B11	-	-	Non-pathogen
B12	-	-	Non-pathogen
B13	+	-	Pathogen
B14	+	-	Pathogen

Notes: (+): causes lysis in blood agar media or necrosis in tobacco leaves; (-): does not cause lysis in blood agar media or necrosis in tobacco leaves

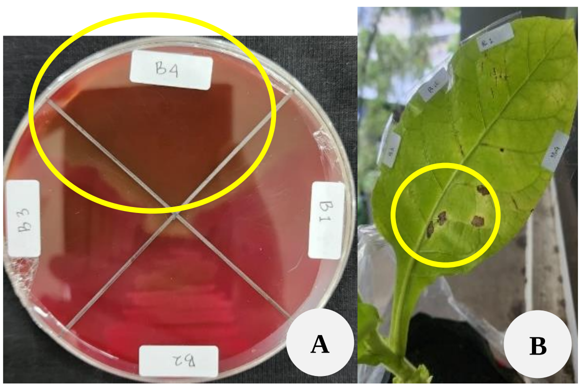

Figure 2. Pathogenicity test of cadmium-resistant bacterial isolates. A. Hemolysis test; B. Hypersensitivity test

Hemolysis assay is considered as a determinant in assessing virulence level and clinical relevance relating to the potential of bacterial isolates as pathogens in humans and animals. In Hemolysis, the compounds produced by bacteria are virulence factors that majorly impact humans and animals (Mogrovejo et al. 2020). The appearance of necrotic symptoms indicates bacterial isolates that cause hypersensitivity reactions. Necrosis symptoms are caused by the formation of antimicrobial secondary metabolites that play a role in blocking the spread of bacterial infection in plant tissues by killing leaf tissue cells induced by bacterial isolates so that pathogenic infections do not spread to other plant tissues (Amaria et al. 2023).

Species identification of cadmium-resistant bacteria

Bacterial species were identified on the three best isolates and not pathogenic, namely isolates B1, B6, and B12. Identification is made by the 16S rDNA sequence method, with the result shown in Figure 3.

The results after phylogentic analysis revealed the identification asisolate B1 has similarities with *Shewanella decolorationis* strain NSSD01 16S ribosomal RNA gene partial sequence with a similarity level of 99.72%, isolate B6 has similarities with *Chryseobacterium cucumeris* strain E1-3 16S ribosomal RNA gene partial sequence with a similarity level of 99.85%, and isolate B12 has similarities with *Aeromonas hydrophila* strain GX5 16S ribosomal RNA gene partial sequence with a similarity level of 99.72%.

Discussion

The high cadmium metal content in four sites 4, 6, 7, and 8 is due to intensive agricultural practices with high use of chemical fertilizers (phonska, NPK, TSP, urea) and insecticides over a long period (more than 50 years) resulting in the accumulation of heavy metal residues. Cd residues come from fertilizers and pesticides containing Cd, such as 0.43 mg/kg in NPK Mutiara, 0.07 mg/kg in urea and 0.51 in SP-36 and 0.04-0.50 mg/kg in several insecticides (Dursban, Emacel, Arjuna, Tripas, Buldok, and Marshal) and fungicides (Antracol, Folicur, Dithane, Octave) (Dewi et al. 2022). Fertilizers with phosphate minerals as the main source contributed 56% of Cd input from anthropogenic activities (Liang et al. 2017).

The location of paddy rice fields near textile factories (sites 4 and 6) is also the cause of high Cd concentrations in the soil at these sites and the use of chemical fertilizers and pesticides. There is a possibility that the irrigation water used on the land is contaminated with textile factory waste, in line with the opinion of Yang et al. (2017) that the main source of Cd contamination in paddy rice farming systems near smelters and mines is irrigation flow contaminated with factory waste. This condition can harm human health because Cd residues can be absorbed by crops and consumed by humans and animals.

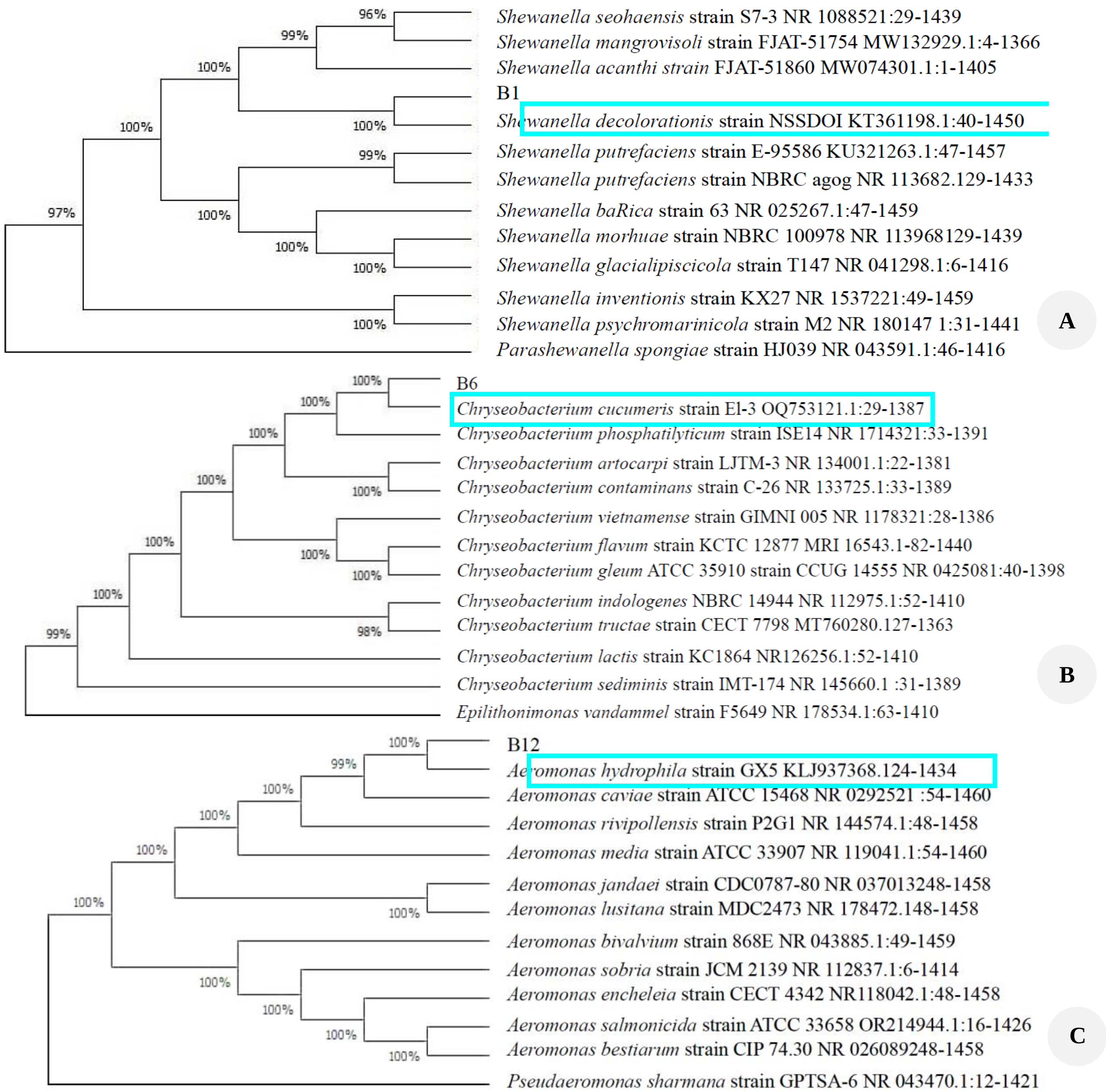

Figure 3. Phylogenic tree based on 16S rRNA sequence. A. Isolate B1; B. Isolate B6; C. Isolate B12

Locations with high Cd content can potentially contain Cd-resistant bacteria because they have adapted to Cd contamination and can grow under Cd stressdue to the presence of an active efflux pump mechanism that removes excess Cd in the cell. Gram-positive bacteria will remove Cd^{2+} through active efflux pump (P-type) transport, while Gram-negative bacteria will undergo the RND (retention, nodulation, division) transport system (Xia et al. 2021). The mechanism of cadmium binding occurs through one or a combination of ion exchange reactions such as complexity, coordination, absorption, electrostatic interaction, chelation, and microprecipitation. Some microorganisms produce carbonate, H_2S, phosphate, and oxalate to react with Cd and form insoluble cadmium salts. In addition, some bacteria produce metal-binding proteins to reduce Cd damage. The urease-producing bacteria will break down urea into carbonate to precipitate Cd ($CdCO_3$) (otavite) by utilizing the urease enzyme it produces. Some sulfate-reducing bacteria produce H_2S to form CdS precipitates (greenockit and hawleyite), intracellularly or extracellularly. Some bacteria can increase the soil's soluble phosphate and phosphoric acid content, potentially incorporating phosphate with Cd to form insoluble cadmium phosphate (Bravo and Brassant 2021; Xia et al. 2021).

Bacteria can be utilized in the bioremediation process because of their bioaccumulation ability. The mechanism of different Cd concentration levels, emphasizes the role of intra-cellular and extra-cellular sequestration in bioaccumulation. That question was explained by the results of Huang et al. (2014) at lower Cd concentrations (below 20 mgL^{-1}), initially adsorbed on the cell surface. Some metal ions were transported and sequestered in the cytoplasm, where there are certain unknown components involved in Cd binding. In contrast, at higher concentrations (above 20 mgL^{-1}), once the metal was transferred into the cytoplasm. The growing cells will start to operate an energy-dependent depletion system to remove excessive amounts of cadmium ions from the cytoplasm, which will protect the cells from highly toxic Cd. The depletion mechanism might function at a certain metal concentration, e.g. 20 mgL^{-1}, to keep intracellular Cd levels below the toxic threshold.

There are 3 best Cd-resistant bacteria isolates from 14 bacteria isolates found with different genera, namely *S. decolorationis* strain NSSD01, *C. cucumeris* strain E1-3, and *A. hydrophila* strain GX5. The *Shewanella* genus is a bacterium that is widely used as an environmental bioremediator because this genus can reduce organic and inorganic components including metal contaminants in the soil. *S. decolorationis* is a Gram-negative bacterium that can live at temperatures of 4-40°C with an optimum temperature ranging from 20-30°C and pH ranging from 7.0-10 with an optimum pH of 8.0. *S. decolorationis* can produce H_2S from thiosulfate and can reduce nitrate, nitrite, ferric materials, and thiosulfate with lactate or acetate as electron donors (Lemaire et al. 2019; Wang et al. 2021).

Chryseobacterium cucumeris (B6) is a yellow Gram-negative bacterium found in the roots of cucumber plants (Jeong et al. 2017), by the isolation location which is used as a rice field with cucumber crop rotation. These bacteria can live at temperatures of 10-38°C with an optimum temperature of 25°C and can survive at pH 5.0-8.0 with an optimum pH of 6.0-8.0. Test results using API 20E kit (bioMerieux) *C. cucumeris* can produce β-galactosidase, urease, gelatinase, and indole (Jeong et al. 2017). Jung et al. (2023) also showed that *C. cucumeris* could be utilized as PGPB (plant growth promoting bacteria) because it produces Indole-3-Acetic Acid (IAA) and plays a role in the nitrogen cycle (has genes in controlling nitrogen metabolism, ammonium transporter, nitrite reduction, and nitric oxide reduction), phosphate (phosphate transporter and polyphosphate metabolism) sulfur (sulfate transporter and sulfur metabolism) and zing (zinc transporter). *A. hydrophila* (B12) species are Gram-negative bacteria, rod-shaped, round colonies and white to dark yellow (Arwin et al. 2016), per the results of morphological identification of B12 devotion. *A. hydrophila* can live in a wide temperature range, which ranges from 4-40°C. This bacterial species can produce indole from the amino acid tryptophane through the enzyme tryptophanase and produce H_2S (Arwin et al. 2016). The results of research by Awan et al. (2018) showed that some *A. hydrophila* strains play a role in phosphate (PO_4^{3-}) regulation, Mg^{2+} transport, N_2 regulation, and K^+ transport.

In conclusion, from 4 places with Cd content >0.5 ppm, 14 Cd-resistant bacterial isolates with different levels of resistance were obtained. Cadmium-resistant bacteria can grow well at cadmium concentrations <20 ppm and begin to be inhibited at concentrations >50 ppm. The three best isolates were found with the highest level of resistance and not pathogenic, namely isolates B1, B6, and B12, which were successively identified as *S. decolorationis* strain NSSD01, *C. cucumeris* strain E1-3, and *A. hydrophila* strain GX5. These three isolates have the potential to be used as bioremediators of Cd metal in soil because they have a high level of resistance and can grow well in environments with high cadmium stress.

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