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Pantoea agglomerans, *Klebsiella pneumoniae*, and *Shigella flexneri* isolated from the Cisadane River as multiresistant bacteria to copper and dyes

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ABSTRACT Copper pollution in Cisadane is a serious environmental issue that needs to be resolved immediately due to its negative impacts on river ecosystems. Bioremediation utilising indigenous bacteria offers excellent potential to restore copper-contaminated river water. This study aimed to obtain indigenous copper-resistant bacteria isolated from the Cisadane River as copper bioremediation agents. Bacteria from Cisadane River water samples were isolated by the spread plate method on Luria Bertani medium containing 3 mM $CuSO_4$. Resistance was determined based on the minimum inhibitory concentration value, while copper concentration was measured using an atomic absorption spectrophotometer. The results presented a total of 13 bacterial isolates with a minimum inhibitory concentration of up to 8 mM $CuSO_4$. Sequence alignment analysis was performed on three selected copper-resistant bacteria, i.e. isolate IrCis1, IrCis4 and IrCis13, which were identified as *Pantoea agglomerans*, *Klebsiella pneumoniae* and *Shigella flexneri* based on 16S rRNA, respectively. Each isolate accumulated copper at 1.19 mg, 1.34 mg and 0.92 mg/g DW of cells, with copper biosorption potentials of 73.74%, 70.17% and 67.73%, respectively. In conclusion, *P. agglomerans* strain IrCis1, *K. pneumoniae* strain IrCis4 and *S. flexneri* strain IrCis5 isolated from the Cisadane River can be used as copper bioremediation agents.

KEYWORDS accumulation; biosorption; Cisadane isolates; copper; resistance

1. Introduction

Cisadane River is a river that flows across Bogor and Tangerang Regencies with an overall length of approximately 126 km. Cisadane River largely influences the livelihoods of people living around the watershed as it is widely used as a main water source for household activities, agriculture, fishing, or other industrial activities (Dawud et al. 2016). However, the discharge of domestic waste into the river has significantly decreased the Cisadane River's water quality (Siahaan et al. 2011; Dawud et al. 2016). Rochyatun et al. (2010) reported that the discharge of domestic waste into the Cisadane River becomes a serious problem because it contaminates toxic chemicals such as cadmium, arsenic, chromium, lead, zinc, nickel and copper (Rochyatun et al. 2010). Sample collected from Cisadane river water analysed for copper content showed relatively high content at 0.13 mg/L, which is higher than the maximum limit allowed: 0.02 mg/L (Irawan et al. 2016). This is exacerbated by improper governance and a lack of concern and awareness of the local community to maintain the sustainability of the river (Dawud et al. 2016).

Copper is a heavy metal widely found in waste due to industrial activities. Excessive amounts of copper in the body of organisms will be hazardous because it can cause toxic effects such as carcinogenicity (Ahmad et al. 2019). Copper can interfere with metabolic function in humans and cause damage or loss of function of vital organs such as heart, brain, kidneys, and liver as well as even cause cancer and death (Aktas 2019). Copper is also a material that cannot be degraded yet easily sedimented in soils, hence easy to accumulate in the food chain (Sarma et al. 2011). Copper contamination in rivers that exceed the threshold can disrupt the natural balance in an ecosystem, reduce the biodiversity of river organisms, and even cause harm towards human health (El Baz et al. 2015).

Copper pollution in river water needs to be taken seriously in anticipation of severely negative impacts on river ecosystems as well as the lives of people living around watersheds. Copper waste treatment methods have been widely developed with physical and/or chemical approaches such as deposition, membrane filtration, ion exchange, reduction-oxidation, neutralisation, or coagula-

tion (Donde 2017; Ahmad et al. 2019; Sarma et al. 2011). However, copper waste treatment methods with physical or chemical approaches are ineffective and have disadvantages such as high-cost budgets and some methods of producing by-products that are toxic to the environment. Waste treatment methods with biological approaches can be an alternative solution because it is more economical, practical, and environmentally friendly (Fidiastuti and Suarsini 2017; Irawati et al. 2019). The most popular conventional technology for removing heavy metal ions is chemical deposition which unfortunately comes with the disadvantage of producing waste sludge and activated carbon and requires ion exchange resins made from unsustainable non-renewable sources. Using bacterial biomass as a bioremediation agent that acts as a renewable resource is an alternative method for removing heavy metals from the environment through bioaccumulation and biosorption processes. Bioaccumulation is a natural biological phenomenon in which microorganisms use proteins to pick up and store metal ions in the intracellular space for use in cellular processes (Diep et al. 2018).

Bioremediation, a natural and cost-effective process utilising indigenous bacteria, can potentially restore copper-contaminated river water. Indigenous bacteria are eco-friendly alternatives with great potential to be utilised as bioremediation agents. Advantages of utilising indigenous bacteria include its natural obtaining process that can be done by isolating a certain type of waste which is then purely cultured through an *in vitro* process in the laboratory, followed by being repeatedly used as starters to process waste in polluted environments (Fidiastuti and Suarsini 2017). According to Kang et al. (2016), bacteria can grow in environments contaminated with heavy metals as they have a resistance that comes from producing compounds to absorb various types of heavy metals, including copper, so that they can be used as biosorbents and bioaccumulators of heavy metals. The results of bacterial metabolism are known to convert harmful substances into environmentally friendly by-products (Yetunde Mutiat et al. 2018; Tahya et al. 2019).

Previous studies reported several indigenous copper-resistant bacteria that have been isolated from copper-contaminated areas, such as *Streptomyces* and *Amycolatopsis* genera (El Baz et al. 2015), *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter* sp. (Irawati et al. 2019), *Alcaligenes faecalis*, *Rhizobium halophytocola*, *Proteus vulgaris*, *Serratia* sp., and *Pseudomonas* (Irawati et al. 2020b). Exploration of copper resistant bacteria from contaminated locations is still being carried out to find indigenous bacteria that have the potential to be used as copper bioremediation agents. This study aimed to isolate, characterise, and examine the potential of indigenous bacteria from the Cisadane River as copper bioremediation agents. This research includes (1) isolation and characterisation of copper-resistant bacteria, (2) determination of bacterial resistance, (3) molecular identification, and (4) determination of accumulation and biosorption potential. The results of the study are expected to be valuable information in the exploration of the potential biological wealth of Indonesian indigenous bacteria as bioremediation agents, as well as contribute to the advancement of science and technology in the field of environment and biotechnology.

2. Materials and Methods

2.1. Isolation and characterisation of copper-resistant bacteria

Copper-resistant bacteria were isolated by spreading on Luria Bertani (LB) media containing copper sulphate ($CuSO_4$). The stock solution used was 1000 mM $CuSO_4$ dissolved in sterile water and sterilised with an autoclave at 121 °C, with a pressure of 15 psi for 15 min. Cisadane River water samples were dissolved in sterile water with a dilution factor of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. Each suspension of 100 µL was taken by micropipette and spread into LB solid media containing 3 mM $CuSO_4$ by Glass Rod Spreader Pyrex and then incubated at 37 °C for 24 h. Colonies that grew were selected and purified until a single colony was obtained. Bacterial isolate purification was done by taking visible separate colonies using an ose needle that was then scratched onto a petri dish containing LB solid media with an added concentration of 3 mM $CuSO_4$, then incubating at 37 °C for 24 h. For further research, a single colony was inoculated on a tilt solid medium containing 3 mM $CuSO_4$ (Irawati et al. 2019).

2.2. Determination of Bacterial Resistance to Copper

Bacterial resistance testing to copper was conducted by determining the minimum inhibitory concentration (MIC) value gradually in the LB solid medium containing various concentrations of $CuSO_4$ by streaking plate methods until no colony grew on the medium. The specified MIC value was determined after 48 h of incubation at 37 °C. Copper resistance tests need to be performed on isolate bacteria that are successfully isolated to find out the maximum resistance of bacteria to heavy metals such as copper (Mazalan et al. 2020). Resistance tests on bacteria isolated from the Cisadane River were carried out in several stages. Bacteria that survive at a concentration of 3 mM will be moved to a medium with a higher concentration of 4 mM, then bacteria capable of growing at a concentration of 4 mM will be moved to a medium with a concentration of 5 mM. The resistance test was continued to a higher copper concentration until the bacteria no longer showed any growth. Each test medium was replicated three times.

2.3. Molecular identification of bacterial isolates

The three selected bacterial isolates were grown in liquid LB medium temperature of 37 °C and stirring speed of 200 rpm for 20 h. Furthermore, bacterial cells were harvested and identified based on the gene 16S rRNA which refers to the naming of bacterial species in GenBank. Gen 16S rDNA was amplified using the Polymerase Chain Reaction method. Forward and reverse Primers used were

the conserved areas of the gene 16S rRNA in *Escherichia coli*. The DNA Polymerase tag used is Plantinum® The DNA Polymerase Tag with a concentration of 5 units/µL and the final concentration of enzymes per reaction of 1.25 units/25 µL.

2.4. Determination of accumulation and biosorption potential

The starter culture was created by inoculating one ose of colony culture into 5 mL of liquid medium, then incubated in the incubator at 37 °C until it reached the logarithm phase. The starter culture (250 µL) was inoculated into 25 mL of liquid LB medium containing various concentrations of $CuSO_4$. The culture was incubated at 37 °C with shaking at 200 rpm. Bacterial growth was observed by measuring cell turbidity (optical density/OD) using a LaboMed spectrophotometer at 600 nm. Bacterial culture was harvested by centrifugation at 4500 rpm for 10 min to separate the cell and medium parts. Harvested cells that had been determined their dry weight and their medium were objects for acid destruction by using HNO_3 and heating at 110 °C to determine the potential accumulation and biosorption of copper. Each sample was replicated three times. Copper analysis was done using Atomic Absorption Spectrophotometer.

3. Results and Discussion

3.1. Copper-resistant bacteria isolation results

The isolation of bacteria from Cisadane was carried out on LB solid media with the lowest $CuSO_4$ concentration of 3 mM. Cisadane bacterial isolation results obtained 13 isolates coded as IrCis1, IrCis2, IrCis3, IrCis4, IrCis5, IrCis6, IrCis7, IrCis8, IrCis9, IrCis10, IrCis11, IrCis12 and IrCis13. The characterisation of bacteria was carried out by observing the colour of the colony, its optical appearance, and the edge of the colony. Gram staining was done to identify cell shape as well as bacterial cell wall type (Table 1).

The colony morphologies of Cisadane bacterial isolates differed, with white as a dominant colony colour. All bacterial isolates were coccus with Gram-negative staining results. This finding was similar to several previous research results, which reported the most indigenous copper-resistant bacteria to be Gram-negative bacteria with high tolerance to copper and heavy metals biosorption capability (Cismasiu 2011; Neethu et al. 2015; Alam and Imran 2017; Giovanella et al. 2017). A large number of Gram-negative bacteria isolates indicated the transfer of plasmids from copper-resistant bacteria in nature, such as *Pseudomonas syringae* and *Escherichia coli* which had plasmids containing copper-resistant genes (Bondarczuk and Piotrowska-Seget 2013). The plasmids found in these two bacteria were easily transmitted between Gram-negative bacteria in natural environments, resulting in many Gram-negative bacteria that are resistant to copper.

Another report has shown that several Gram-negative bacteria have higher heavy metal biosorption capacity than Gram-positive (Kurnia et al. 2015; Alam and Imran 2017). As stated by Kapahi and Sachdeva (2019), biosorption efficiency depends on bacterial species and cell wall structure containing peptidoglycans such as N-acetylmuramic acid and poly-N-acetylglucosamine. The cell wall of bacteria contains a negative charge which attracts and connects with metal ions. The negative charge in Gram-negative bacteria is found in peptidoglycan, lipopolysaccharides, and phospholipids which determine the metal binding capacity of bacteria.

3.2. Copper resistance test results

Thirteen bacterial isolates isolated from the Cisadane river were able to grow up in a medium containing 6 mM $CuSO_4$ while at a concentration of 7 mM and 8 mM, only nine isolates were able to grow, i.e., IrCis1, IrCis2, IrCis4, IrCis5, IrCis6, IrCis7, IrCis9, IrCis10 and IrCis13 (Table 2).

Copper is a trace element required by many microorganisms, yet it can exert an inhibitory effect on several

TABLE 1 Morphological characterisation of Cisadane bacterial isolate colonies

No	Isolate code	Color of colony	Optical appearance	Edge of colony	Cells shape	Gram staining
1	IrCis1	White	Transparent	Jagged	Coccus	Negative
2	IrCis2	Pale Yellow	Opaque	Smooth	Coccus	Negative
3	IrCis3	Yellow	Opaque	Smooth	Coccus	Negative
4	IrCis4	White	Opaque	Smooth	Coccus	Negative
5	IrCis5	White	Transparent	Jagged	Coccus	Negative
6	IrCis6	White	Transparent	Smooth	Coccus	Negative
7	IrCis7	White	Transparent	Irregular	Coccus	Negative
8	IrCis8	Pale Yellow	Transparent	Irregular	Coccus	Negative
9	IrCis9	White	Transparent	Jagged	Coccus	Negative
10	IrCis10	Yellow	Opaque	Smooth	Coccus	Negative
11	IrCis11	Pale Yellow	Opaque	Smooth	Coccus	Negative
12	IrCis12	Pale Yellow	Opaque	Jagged	Coccus	Negative
13	IrCis13	White	Opaque	Jagged	Coccus	Negative

TABLE 2 Resistance tests results of bacteria isolated from the Cisadane river

No	Isolate code	Resistance test with variation of $CuSO_4$ (mM) concentration						
		3 mM	4 mM	5 mM	6 mM	7 mM	8 mM	9 mM
1	IrCis1	+	+	+	+	+	+	-
2	IrCis2	+	+	+	+	+	+	-
3	IrCis3	+	+	+	+	-	-	-
4	IrCis4	+	+	+	+	+	+	-
5	IrCis5	+	+	+	+	+	+	-
6	IrCis6	+	+	+	+	+	+	-
7	IrCis7	+	+	+	+	+	+	-
8	IrCis8	+	+	+	+	-	-	-
9	IrCis9	+	+	+	+	+	+	-
10	IrCis10	+	+	+	+	+	+	-
11	IrCis11	+	+	+	+	-	-	-
12	IrCis12	+	+	+	+	-	-	-
13	IrCis13	+	+	+	+	+	+	-

Note: + : Grow, - : Does not grow

bacterial growths at relatively low concentrations. However, several bacterial species can tolerate high levels of copper, and the highest concentration varies among species. Moreover, different bacteria have adopted diverse strategies for copper resistance. In this study, we observed that at a concentration of 9 mM $CuSO_4$, there was no visible growth among thirteen isolates. This observation indicated that copper has become very toxic to isolated bacteria at a concentration of 9 mM (Fowler et al. 2019). Several isolated could resist copper at a maximum concentration of 8 mM. Biswas (2015) stated that bacteria could utilise heavy metals as a cofactor for enzymes involved in energy production (Biswas 2015). Some metals such as chromium, calcium, magnesium, manganese, copper, sodium, nickel and zinc become sources of micronutrients which supports metabolic function. Metabolism can be performed by bacteria degrading wastes by producing certain enzymes such as oxygenises and electron transport proteins (Fidiastuti and Suarsini 2017).

3.3. Growth of bacteria on medium supplemented with various concentrations of copper

Bacterial isolates from the Cisadane River could grow on a medium supplemented with various concentrations of copper with a range of 5 mM - 7 mM. Streaked bacterial growth in a medium containing 5 mM copper was less than that of at 7 mM (Figure 1). The reduction of bacterial growth at higher copper concentrations indicates that copper in the growth medium may be one of the factors inhibiting bacterial growth (Mazalan et al. 2020; Irawati et al. 2019). The growth of bacteria in a medium with copper content at different concentrations provides evidence that isolated bacteria have a cell mechanism for copper resistance.

According to Neethu et al. (2015), microorganisms living in high-concentration heavy-metal environments develop different mechanisms to adapt to these heavy-metal stresses, such as adaptation mechanisms, enzymatic oxidation or reduction to a less toxic form, metal sorption, mineralisation, uptake and accumulation, extracellular precipitation, and efflux of heavy metals from the cells. Previous studies show that copper-resistant bacteria conducted a mechanism encoded plasmid to prevent the absorption of free copper ions into cells. On the other hand, a number of studies revealed that some copper resistance mechanisms were encoded by chromosomes for the absorption and management of low-concentration copper (Bondarczuk and Piotrowska-Seget 2013). While carrying out resistance mechanisms, bacteria can bind, mobilise, and decrease the toxicity of heavy metals in order to achieve bioremediation goals. Heavy metal pollutants cannot be degraded, thus, the principle of microbial remediation of the environment contaminated with heavy metals mainly includes biosorption, bioaccumulation, and bioconversion to a non-toxic form. Bacteria utilise the negative side of the cell surface to bind heavy metal ions through adsorption or electrostatic complexation, store toxic metals absorbed in various parts of cells, or bind it to the extracellular matrix (Park and Chon 2016).

3.4. The ability of copper biosorption and accumulation

The potential of bacterial isolates as copper bioremediation through biosorption and accumulation processes was observed in IrCis1, IrCis4, and IrCis5. These bacterial isolates were randomly selected as representatives of the three highest resistant bacteria with an MIC of 8 mM $CuSO_4$. Sequence alignment analysis showed that IrCis1, IrCis4, and IrCis5 were designated as *Pantoea agglomerans*, *Klebsiella pneumoniae*, and *Shigella flexneri* with similarity of 97.75%, 97.26%, and 96.95%, respectively (Table 3).

The ability of *P. agglomerans* strain IrCis1, *K. pneumoniae* strain IrCis4, and *S. flexneri* strain IrCis5 to accu-

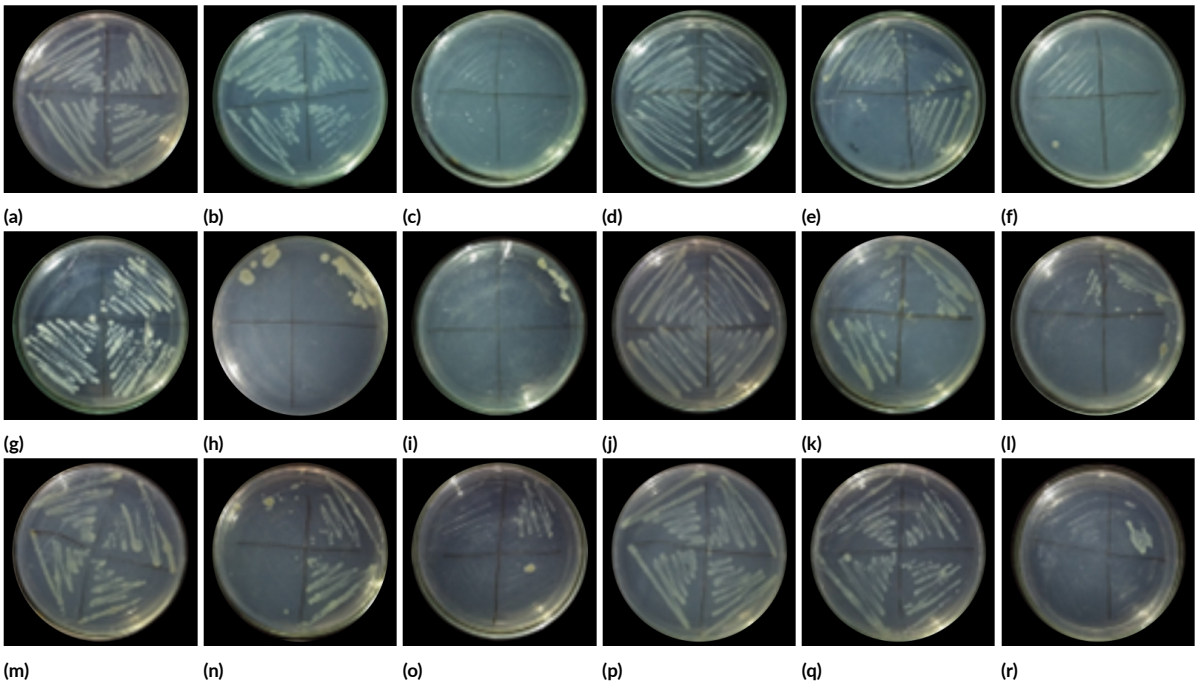

FIGURE 1 Growth of Cisadane bacterial isolates in a medium containing various concentrations of $CuSO_4$. **a**: IrCis1 in 5 mM, **b**: IrCis1 in 6 mM, **c**: IrCis 1 in 7 mM. **d**: IrCis3 in 5 mM, **e**: IrCis3 in 6 mM, **f**: IrCis3 in 7 mM. **g**: IrCis4 in 5 mM, **h**: IrCis4 in 6 mM, **i**: IrCis4 in 7 mM. **j**: IrCis5 in 5 mM, **k**: IrCis5 in 6 mM, **l**: IrCis5 in 7 mM. **m**: IrCis6 in 5 mM, **n**: IrCis6 in 6 mM, **o**: IrCis6 in 7 mM. p: IrCis9 in 5 mM, **q**: IrCis9 in 6 mM, **r**: IrCis9 in 7 mM.

mulate copper were 0.70 mg/g, 0.73 mg/g, and 0.77 mg/g in medium containing 2 mM $CuSO_4$ which increased to 1.19 mg/g, 1.34 mg/g, and 0.92 mg/g in medium supplemented with 4 mM $CuSO_4$, respectively (Figure 2). The accumulation ability of this Cidasane bacterial isolates was greater than that of *Enterobacter cloacae* IrSuk1 strain, *Enterobacter cloacae* strain IrSuk4a, and *Serratia nematodiphila* strain IrSuk13 with copper accumulation abilities of 0.45 mg, 0.74 mg, and 1.86 mg copper per gram dry weight of cells (Irawati and Tahya 2021) and *Acinetobacter* sp. IrC2 with copper accumulation ability of 0.23 mg per gram dry weight of cells (Irawati et al. 2020a).

The ability of copper accumulation in *P. agglomerans* strain IrCis1 increased according to the increase in copper concentration from 2 mM to 4 mM, namely 0.70 mg and 1.19 mg, respectively. The accumulation ability of this bacterial strain was higher than that of *P. agglomerans* isolated from contaminated land in Abare, Nigeria, with an accumulation ability of 0.234-0.177 mg (Audu et al. 2020). The results of this study indicate that bacteria isolated from the environment-contaminated copper can accumulate copper as a form of resistance mechanism so that they have the potential to remove copper from the environment. This is in line with the results of the research of Paulina et al. (2014), who reported that the *P. agglomerans* strain LMAE-2 isolated from sediments of the Tenglo channel, Puerto Montt, Chile was proven to have copper resistance. This phenomenon is supported by Acioly et al. (2018), who reported that *P. agglomerans* are equipped with high resistance to copper and copper-removal abilities.

TABLE 3 Resistance tests results of bacteria isolated from the Cisadane river.

Code of sample	Identified species	Accession Number	Similarity (%)
IrCis1	*Pantoea agglomerans* strain IrCis1	MW521823	97.75
IrCis4	*Klebsiella pneumoniae* strain IrCis4	MW532150	97.26
IrCis5	*Shigella flexneri* strain IrCis5	MW532149	96.95

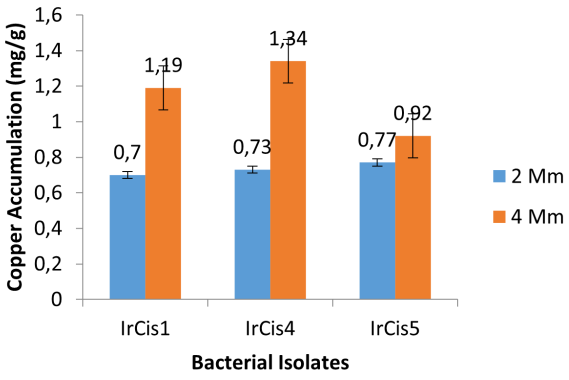

FIGURE 2 Ability of three Cisadane bacterial isolates in accumulating copper on medium with 2 mM and 4 mM $CuSO_4$. Error bars represent standard error from the mean of triplicates measurement.

The ability of *Klebsiella pneumoniae* strain IrCis4 to accumulate copper in a medium containing 2 mM of $CuSO_4$ was 0.73 mg which increased to 1.34 mg in a medium containing 4 mM. The copper accumulation capability of this bacterial strain was lower than that of *K. pneumoniae* CN1 and CN6 isolated from Cikapundung, with accumulation abilities of 4.62 mg/g and 3.12 mg/g dry weight of cells, respectively (Irawati et al. 2019). Results showed that the same bacterial species could have different copper accumulation abilities depending on the level of contamination where the bacteria are isolated. Reports from Abbas et al. (2014) who has previously succeeded in isolating *K. pneumonia* from industrial waste, further elucidate the resistance of this bacteria strain to copper.

In this study, the ability of Shigella flexneri strain IrCis5 to accumulate copper was 0.77 mg/g in a medium containing 2 mM $CuSO_4$ and increased to 0.92 mg/g in a medium supplemented with 4 mM $CuSO_4$. Not many studies have reported the ability of this bacterial species to accumulate copper, but Noman et al. (2020) successfully isolated *S. flexneri* strain SNT22 from soil contaminated with wastewater which is thought to have the ability to accumulate copper.

Figure 3 shows that the copper biosorption ability of *P. agglomerans* strain IrCis1 and *S. flexneri* strain IrCis5 increased along with the increase of copper concentration. Copper biosorption ability of *P. agglomerans* strain IrCis1 in medium containing 2 mM and 4 was 71.49% and 73.74% was higher than that of *P. agglomerans* isolated from contaminated land in Abare, Nigeria (Audu et al. 2020) and *P. agglomerans* LMAE-2 isolated from marine sediments (Paulina et al. 2014) with the value of 60% and 11.6%, respectively. Further studies indicated that *P. agglomerans* LMAE-2 had cop genes encoded copper homeostasis and resistance (Corsini et al. 2016).

The ability of *S. flexneri* strain IrCis5 to biosorp copper in medium 2 mM and 4 mM was 66.76% and 67.73%, respectively. Noman et al. (2020) reported that *S. flexneri* SNT22 had copper-resistance properties. According to El-Sherbiny and Shehata (2014), *Shigella* sp. had a detoxification mechanism developed by other resistant microorganisms, such as complexation by exopolysaccharides, binding with bacterial cell envelopes, metal reduction, and metal efflux. A previous study reported that *S. flexneri* had copper homeostasis CUPCs involved in absorption, storage, shipping, and eflux copper. This mechanism may sometimes encode plasmids that facilitate transfer genes of resistance from one bacterial cell to other cells (Zhu et al. 2005).

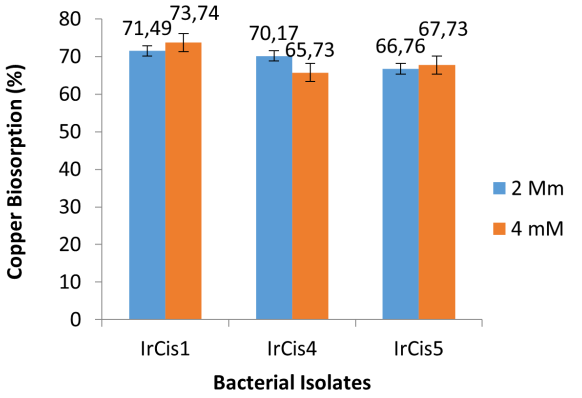

FIGURE 3 Ability of Cisadane bacterial isolates in performing copper biosorption on medium containing 2 mM and 4 mM $CuSO_4$. Error bars represent standard error from the mean of triplicates measurement.

The copper biosorption ability of *K. pneumoniae* strain IrCis4 decreased from 70.17% to 65.73% when the concentration of copper in the medium increased from 2 mM to 4 mM, respectively. The decreasing ability is hypothesised to be caused by the toxicity of copper in high concentrations. The copper biosorption ability of this strain was higher than that of *Klebsiella pneumoniae* strain CN1 and CN6 isolated from Cikapundung, Indonesia, with a value of 36.78% (Irawati et al. 2019). Thus, it can be said that *K. pneumoniae* strain IrCis4 has more significant potential to be utilised as a copper bioremediation agent in future applications.

4. Conclusions

Pantoea agglomerans strain IrCis1, *Klebsiella pneumoniae* strain IrCis4, and *Shigella flexneri* strain IrCis13 were indigenous copper-resistant bacteria isolated from Cisadane River which can accumulate copper at 1.19 mg, 1.34 mg, and 0.92 mg per gram dry weight of cells, and copper biosorption potential of 73.74%, 70.17%, and 67.73%, respectively.

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Authors' contributions

WI designed the study and analysed the data. WI, CYT, G carried out the laboratory work and wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The authors of this article declare no competing interests.

References

Abbas SZ, Rafatullah M, Ismail N, Lalung J. 2014. Isolation, identification, and characterization of cadmium resistant *Pseudomonas* sp. M3 from industrial wastewater . J. Waste Manag. 2014:1–6. doi:10.1155/2014/160398.

Acioly LML, Cavalcanti D, Luna MC, V Júnior JC, S Andrade RF, de Lima e Silva TA, La Rotta CE, Campos-Takaki GM. 2018. Cadmium removal from aqueous solutions by strain of *Pantoea agglomerans* UCP1320 isolated from laundry effluent. Open Microbiol. J. 12(1):297–307. doi:10.2174/1874285801812010297.

Ahmad BS, Hassan T, Majid S. 2019. Heavy metal toxicity and their harmful effects on living organisms-a review. Int. J. Med. Sci. Diagnosis Res. 3(1):106–122. doi:10.32553/JMSDR.

Aktas F. 2019. Bioremediation techniques and strategies on removal of polluted environment. J. Eng. Res. Appl. Sci. 2(1):107–115.

Alam M, Imran M. 2017. Metal tolerance analysis of Gram negative bacteria from hospital effluents of Northern India. J. Appl. Pharm. Sci. 7(4):174–180. doi:10.7324/JAPS.2017.70426.

Audu KE, Adeniji SE, Obidah JS. 2020. Bioremediation of toxic metals in mining site of Zamfara metropolis using resident bacteria (*Pantoea agglomerans*): A optimization approach. Heliyon 6(8):E04704. doi:10.1016/j.heliyon.2020.e04704.

Biswas K. 2015. Biological agents of bioremediation. Front. Environ. Microbiol. 1(3):39–43. doi:10.11648/j.fem.20150103.11.

Bondarczuk K, Piotrowska-Seget Z. 2013. Molecular basis of active copper resistance mechanisms in Gram-negative bacteria. Cell Biol. Toxicol. 29(6):397–405. doi:10.1007/s10565-013-9262-1.

Cismasiu CM. 2011. The adaptation of Gram-negative bacteria to acidic environmental conditions with implication in heavy metals removal processes. Rom. Biotechnol. Lett. 16(6):10–18.

Corsini G, Valdés N, Pradel P, Tello M, Cottet L, Muiño L, Karahanian E, Castillo A, Gonzalez AR. 2016. Draft genome sequence of a copper-resistant marine bacterium, *Pantoea agglomerans* strain LMAE-2, a bacterial strain with potential use in bioremediation. Genome Announc. 4(3):3–4. doi:10.1128/genomeA.00525-16.

Dawud M, Namara I, Chayati N, Taqwa F. 2016. Analisis sistem pengendalian pencemaran air Sungai Cisadane Kota Tangerang berbasis masyarakat. Semin. Nas. Sains dan Teknol. 6(November):1–8.

Diep P, Mahadevan R, Yakunin AF. 2018. Heavy metal removal by bioaccumulation using genetically engineered microorganisms. Front. Bioeng. Biotechnol. 6(OCT):157. doi:10.3389/fbioe.2018.00157.

Donde OO. 2017. Wastewater management techniques: A review of advancement on the appropriate wastewater treatment principles for sustainability. Environ. Manag. Sustain. Dev. 6(1):40. doi:10.5296/emsd.v6i1.10137.

El Baz S, Baz M, Barakate M, Hassani L, El Gharmali A, Imziln B. 2015. Resistance to and accumulation of heavy metals by *Actinobacteria* isolated from abandoned mining areas. Sci. World J. 2015:1–14. doi:10.1155/2015/761834.

El-Sherbiny GM, Shehata ME. 2014. Antimicrobial susceptibility, heavy metals tolerance and plasmid curing of Shigella species isolated from El- Dakahlia, Egypt. Am. J. Microbiol. Res. 2(6):211–216. doi:10.12691/ajmr-2-6-7.

Fidiastuti HR, Suarsini E. 2017. Potensi bakteri indigen dalam mendegradasi limbha cair pabrik kulit secara *in vitro*. Bioeksperimen J. Penelit. Biol. 3(1):1. doi:10.23917/bioeksperimen.v3i1.3665.

Fowler L, Engqvist H, Öhman-Mägi C. 2019. Effect of copper ion concentration on bacteria and cells. Materials (Basel). 12(22):1–13. doi:10.3390/ma12223798.

Giovanella P, Cabral L, Costa AP, de Oliveira Camargo FA, Gianello C, Bento FM. 2017. Metal resistance mechanisms in Gram-negative bacteria and their potential to remove Hg in the presence of other metals. Ecotoxicol. Environ. Saf. 140:162–169. doi:10.1016/j.ecoenv.2017.02.010.

Irawan DE, Puradimaja DJ, Yeni D, Kuntoro AA, Julian MM. 2016. Decreasing groundwater quality at Cisadane riverbanks: groundwater-surface water approach. arXiv: Geophysics p. 1–7. doi:10.48550/arXiv.1603.09380.

Irawati W, Hasthosaputro A, Kusumawati L. 2020a. Multiresistensi dan akumulasi *Acinetobacter* sp. IrC2 terhadap logam berat. J. Biol. Papua 12(2):114–122. doi:10.31957/jbp.1207.

Irawati W, Ompusunggu NP, Susilowati DN, Yuwono T. 2019. Molecular and physiological characterization of indigenous copper-resistant bacteria from Cikapundung River, West Java, Indonesia. Biodiversitas 20(2):344–349. doi:10.13057/biodiv/d200206.

Irawati W, Pinontoan R, Yuwono T. 2020b. Indigenous copper resistant bacteria isolated from activated sludge of water treatment plant in Surabaya, Indonesia. Biodiversitas 21(11):5077–5084. doi:10.13057/biodiv/d211112.

Irawati W, Tahya CY. 2021. Copper removal by *Enterobacter cloacae* strain IrSuk1, *Enterobacter cloacae* strain IrSuk4a, and *Serratia nematodiphila* strain IrSuk13 isolated from Sukolilo River-Indonesia. IOP Conf. Ser. Mater. Sci. Eng. 1053(1):1–9. doi:10.1088/1757-899x/1053/1/012038.

Kang CH, Kwon YJ, So JS. 2016. Bioremediation of heavy metals by using bacterial mixtures. Ecol. Eng. 89:64–69. doi:10.1016/j.ecoleng.2016.01.023.

Kapahi M, Sachdeva S. 2019. Bioremediation options for heavy metal pollution. doi:10.5696/2156-9614-9.24.191203.

Kurnia K, Sadi NH, Jumianto S. 2015. Isolation and characterization of Pb resistant bacteria from Cilalay Lake, Indonesia. Aceh Int. J. Sci. Technol. 4(3):83–87. doi:10.13170/aijst.4.3.3016.

Mazalan NZS, Oyeleye A, Rahman RNZRA, Aris AZ, Salleh AB, Normi YM. 2020. Isolation and characterization of an acid and metal tolerant *Enterobacter cloacae* NZS strain from former mining lake in Selangor, Malaysia. Beni-Suef Univ. J. Basic Appl. Sci. 9(1):1–12. doi:10.1186/s43088-020-00051-1.

Neethu CS, Rahiman KM, Saramma AV, Hatha AA. 2015. Heavy-metal resistance in gram-negative bacteria isolated from kongsfjord, Arctic. Can. J. Microbiol. 61(6):429–435. doi:10.1139/cjm-2014-0803.

Noman M, Ahmed T, Hussain S, Niazi MBK, Shahid M, Song F. 2020. Biogenic copper nanoparticles synthesized by using a copper-resistant strain *Shigella flexneri* SNT22 reduced the translocation of cadmium from soil to wheat plants. J. Hazard. Mater. 398:123175. doi:10.1016/j.jhazmat.2020.123175.

Park JH, Chon HT. 2016. Characterization of cadmium biosorption by *Exiguobacterium* sp. isolated from farmland soil near Cu-Pb-Zn mine. Environ. Sci. Pollut. Res. 23(12):11814–11822. doi:10.1007/s11356-016-6335-8.

Paulina P, Corsini G, Mario T, González A. 2014. *Pantoea agglomerans* an agent to remove residual copper from aquaculture activity. Adv. Mater. Res. 945-949:3479–3482. doi:10.4028/www.scientific.net/AMR.945-949.3479.

Rochyatun E, Kaisupy MT, Rozak A. 2010. Distribusi logam berat dalam air dan sedimen di perairan muara Sungai Cisadane. MAKARA Sci. Ser. 10(1):35–40. doi:10.7454/mss.v10i1.151.

Sarma H, Deka S, Deka H, Saikia RR. 2011. Accumulation of heavy metals in selected medicinal plants. Rev. Environ. Contam. Toxicol. 214:63–86. doi:10.1007/978-1-4614-0668-6_4.

Siahaan R, Indrawan A, Soedharma D, Prasetyo LB. 2011. Kualitas air Sungai Cisadane, Jawa Barat, Banten. J. Ilm. SAINS 15(1):268. doi:10.35799/jis.11.2.2011.218.

Tahya CY, Irawati W, Purba FJ. 2019. Phenol biodegradation and catechol 2,3-dioxygenase gene sequencing of *Bacillus cereus* IrC2 isolated from rungkut Indonesia. J. Kim. Terap. Indones. 21(1):23–30. doi:10.14203/jkti.v21i1.415.

Yetunde Mutiat FB, Gbolahan B, Olu O. 2018. A comparative study of the wild and mutated heavy metal resistant *Klebsiella variicola* generated for cadmium bioremediation. Bioremediat. J. 22(1-2):1–15. doi:10.1080/10889868.2018.1445695.

Zhu DY, Zhu YQ, Huang RH, Xiang Y, Yang N, Lu HX, Li GP, Jin Q, Wang DC. 2005. Crystal structure of the copper homeostasis protein (CutCm) from *Shigella flexneri* at 1.7 Å resolution: The first structure of a new sequence family of TIM barrels. Proteins Struct. Funct. Genet. 58(3):764–768. doi:10.1002/prot.20362.