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Isolation and Characterization of Copper-Resistant Bacteria *Pseudomonas alcaligenes* CuFr 1.1 and *Pseudomonas chengduensis* CuFr 1.3 from Tembagapura Mine, Papua

Isolasi dan Karakterisasi Bakteri Resisten Tembaga *Pseudomonas alcaligenes* CuFr 1.1 *dan Pseudomonas chengduensis* CuFr 1.3 dari Pertambangan Tembagapura, Papua

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ABSTRAK

Kontaminasi tembaga di tanah merupakan ancaman serius bagi ekosistem dan kesehatan manusia karena efek toksiknya terhadap tanaman, hewan, dan manusia. Salah satu sumber utama pencemaran ini adalah tambang Tembagapura di Papua. Pendekatan yang efektif untuk mengurangi pencemaran tembaga adalah bioremediasi menggunakan bakteri indigenous yang diisolasi dari lingkungan tercemar karena telah beradaptasi dengan kondisi setempat. Penelitian ini bertujuan untuk: (1) mengisolasi bakteri resisten tembaga, (2) mengkarakterisasi morfologi koloninya, (3) menguji resistensi terhadap tembaga, dan (4) mengidentifikasi isolat terpilih berdasarkan gen 16S rDNA. Sampel tanah dikumpulkan dari area tambang Tembagapura dan isolasi bakteri dilakukan menggunakan media Luria Bertani (LB) yang ditambahkan 3 mM $CuSO_4$. Pengujian resistensi dilakukan dengan metode gores (streak plate) untuk menentukan nilai konsentrasi hambat minimum (MIC). Lima isolat bakteri resisten tembaga yang berhasil diperoleh: CuFr 1.1, CuFr 1.2, CuFr 1.3, CuFr 2.1, dan CuFr 2.2. Di antara kelima isolat tersebut, CuFr 1.1 dan CuFr 1.3 menunjukkan resistensi tertinggi dengan nilai MIC mencapai 9 mM. Identifikasi molekuler berdasarkan sekuens gen 16S rDNA menunjukkan bahwa CuFr 1.1 adalah Pseudomonas alcaligenes, sedangkan CuFr 1.3 adalah Pseudomonas chengduensis. Temuan ini merupakan laporan awal mengenai keberadaan bakteri indigenous resisten tembaga dari tambang Tembagapura. Isolat tersebut menunjukkan potensi besar sebagai biosorben tembaga dan kandidat agen bioremediasi pada lokasi yang terkontaminasi. Penelitian lebih lanjut diperlukan untuk mengeksplorasi mekanisme biosorpsi dan efektivitasnya pada skala lapangan.

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ABSTRACT

Copper contamination in soil poses a serious threat to ecosystems and human health due to its toxic effects on plants, animals, and humans. One major source of this pollution is the Tembagapura mine in Papua. An effective approach to mitigate copper pollution is bioremediation using indigenous bacteria isolated from contaminated environments, as they are better adapted to local conditions. This study aimed to: (1) isolate copper-resistant bacteria, (2) characterize their colony morphology, (3) assess their resistance to copper, and (4) identify selected isolates based on the 16S rDNA gene. Soil samples were collected from the Tembagapura mining site, and bacterial isolation was performed using Luria Bertani agar supplemented with 3 mM $CuSO_4$. Resistance testing was conducted using the streak plate method to determine the Minimum Inhibitory Concentration (MIC). Five copper-resistant bacterial isolates were obtained: CuFr 1.1, CuFr 1.2, CuFr 1.3, CuFr 2.1, and CuFr 2.2. Among these, CuFr 1.1 and CuFr 1.3 exhibited the highest resistance, with MIC values reaching 9 mM. Molecular identification using 16S rDNA sequencing revealed that CuFr 1.1 is Pseudomonas alcaligenes, while CuFr 1.3 is Pseudomonas chengduensis. These findings provide the first report of copper-resistant indigenous bacteria from the Tembagapura mine. The isolates show promising potential for development as copper biosorbents and future application as bioremediation agents at contaminated sites. Further research is needed to explore their biosorption mechanisms and performance in field-scale applications.

1. INTRODUCTION

1.1 Background

Heavy metal contamination is an environmental issue that warrants serious attention. Aquatic and terrestrial ecosystems are among the most susceptible to heavy metal pollution (Ali, 2017). One of the toxic heavy metals of concern is copper, which possesses significant redox potential (Burkhead & Collins, 2022). Copper becomes toxic to organisms when present in concentrations exceeding biological tolerance thresholds. The World Health Organization (WHO) has established a permissible average copper concentration of 0.036 m^{3-1} in water and soil. Elevated copper levels in plants can lead to physiological disturbances, thereby inhibiting growth and development (Sağlam *et al*., 2016). Moreover, excessive copper contamination in livestock feed poses a risk to human health through the consumption of animal-derived products (Zhen *et al*., 2022).

One of the primary sources of copper pollution in soil is the Tembagapura mining region in Papua. Previous studies have identified high copper concentrations in soils surrounding this mining area (Ettler *et al*., 2014). Copper contamination in soil may originate from unprocessed mining residues (Boky *et al*., 2015), copper ore tailing storage (Musztyfaga *et al*., 2014), and emissions from copper-nickel industrial complexes (Kashulina, 2017). These sources contribute to the accumulation of copper and other heavy metals in soil, often surpassing safe contamination thresholds and posing environmental and human health risks. The Tembagapura Mining Area in Papua, therefore, represents a potentially significant site of copper pollution.

Copper contamination can be mitigated through various methods, one of which is bioremediation—a proven, environmentally friendly approach. Bioremediation involves the degradation of pollutants by organisms such as bacteria (Puspitasari & Khaeruddin, 2016). Among the most effective agents for bioremediation are indigenous bacteria—microorganisms isolated from contaminated environments that have naturally adapted to pollutant stress *(Lutfi et al.*, 2018). Owing to their adaptation, indigenous bacteria are particularly effective when deployed as bioremediation agents. The use of such bacteria to remediate copper is both cost-effective and environmentally sustainable, as they are reusable and pose minimal ecological disruption (Melati, 2020). Bacteria isolated from contaminated environments have demonstrated the ability to accumulate copper, thereby reducing its concentration in situ (Irawati *et al*., 2022). Several copper-resistant indigenous bacterial strains have been found to perform biosorption, a process through which biological materials absorb and retain heavy metals. Biosorption is simple, economical, and environmentally benign, making it a viable alternative for removing pollutants from the environment (Torres, 2020; Sireesha *et al*., 2022). For example, *Pantoea agglomerans*, *Klebsiella pneumoniae*, and *Shigella flexneri* have demonstrated copper biosorption efficiencies of 73.74%, 70.17%, and 67.73%, respectively (Irawati et al., 2022).

It is therefore hypothesized that indigenous bacteria isolated from copper-contaminated soils in Papua's mining areas may hold significant potential as bioremediation agents for ecological restoration. Copper-resistant bacteria possess unique structural and physiological characteristics that enable survival in high copper concentrations (Rohmayani *et al*., 2021). These microorganisms manage copper toxicity via two main mechanisms: (1) bioaccumulation—the uptake of chemical substances from the environment by living organisms; and (2) biosorption—the passive binding of heavy metals onto biological surfaces. Additionally, these bacteria regulate copper influx to maintain cellular homeostasis (Irawati, 2019).

Numerous studies have reported copper-resistant bacterial strains. For instance, *Sphingomonas* strains O12, A32, and A55, *Stenotrophomonas* strain C21, and *Arthrobacter* strain O4 were isolated from agricultural soils in Chile (Altimira *et al*., 2012). Similarly, strains IrC1, IrC2, and IrC4, isolated from activated sludge at an industrial wastewater treatment plant in Surabaya, Indonesia, were found to be copper-resistant (Irawati *et al*., 2012). According to Zolgharnein *et al*. (2007), bacterial copper resistance is often attributed to the presence of the *copA* gene encoded on plasmids, which enables isolates such as EC38, FC43, and CC53 to grow in media containing 400–500 mg/L of copper(II) sulfate ($CuSO_4$).

Given the environmental and health implications of copper contamination in Papua's mining areas and the prospective utility of indigenous bacteria for bioremediation, it is imperative to isolate and characterize copper-resistant bacterial strains from the Tembagapura Mining Area. This study offers a novel contribution by targeting a sampling site that has not been previously investigated, thereby enhancing the likelihood of discovering highly potent and beneficial bacterial candidates for sustainable bioremediation applications.

1.2 Research Objectives

This study aims to isolate copper-resistant bacteria from mining soils in Papua, characterize the colony morphology of the bacterial isolates, assess their resistance to copper, and identify the isolates based on 16S rDNA gene sequencing. The identification process includes taxonomic classification of the isolates and the construction of a phylogenetic tree to determine their evolutionary relationships.

2. METHODOLOGY

2.1 Isolation of Copper-Resistant Bacteria

The medium used for the isolation and characterization of bacteria was Luria Bertani agar (LB agar), an enriched medium suitable for the growth of a wide range of bacterial species (Tumbel *et al*., 2017). LB agar contains yeast extract as a nutrient source, sodium chloride to maintain osmotic balance, glucose as a carbon source, and agar as a coagulating agent to support bacterial growth (Darokar, n.d.). Copper was introduced in the form of copper sulfate ($CuSO_4$) at a stock concentration of 1 molar to select for copper-resistant bacteria. The $CuSO_4$ stock was added to the sterilized medium at the desired concentration. The sample used in this study was soil collected from the Tembagapura mining area in Papua. The bacterial sample was diluted with 50 mL of sterile distilled water (dH_2O). A 100 µL aliquot of the serially diluted suspension was inoculated onto LB agar medium containing 3 mM $CuSO_4$ and incubated at 37 °C for 72 hours. Colonies

that grew were selected and assigned identification codes. The resulting bacterial isolates were then purified to obtain pure cultures. Purification was conducted by streaking the colonies onto fresh LB agar plates supplemented with 3 mM $CuSO_4$, followed by incubation at 37 °C for 24 hours. The pure cultures were subsequently preserved on slant media containing 3 mM $CuSO_4$ (Irawati, 2019).

2.2 Morphological Characterization of Copper-Resistant Bacterial Colonies

The morphological characteristics of the copper-resistant bacterial colonies were assessed based on colony shape, surface texture, margin type, optical properties, and pigmentation. The Gram reaction (positive or negative) and cell morphology were determined through Gram staining and microscopic observation using a binocular microscope at 1000x magnification (Irawati *et al*., 2022).

2.3 Copper Resistance Testing of Bacteria

Resistance testing was conducted to determine the minimum inhibitory concentration (MIC) of each bacterial isolate against copper. This test identifies the lowest concentration of $CuSO_4$ that inhibits bacterial growth. The MIC test was carried out using LB agar media supplemented with $CuSO_4$ at concentrations of 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, and 8 mM. Bacterial isolates were streak-inoculated onto LB agar plates containing increasing concentrations of $CuSO_4$. The plates were then incubated at 37 °C for 48 hours. Isolates that exhibited growth were subsequently inoculated onto media with higher copper concentrations until the minimum concentration at which bacterial growth was inhibited (i.e., MIC) was established (Irawati *et al*., 2022).

2.4 Identification of Bacterial Isolates Based on 16S rDNA Gene

Bacterial isolate identification was performed by amplifying the 16S rDNA gene and sequencing the nucleotide bases to assess sequence similarity using gene bank data. Sequence alignment was conducted using Clustal X version 2.0 with the neighbor-joining method, and phylogenetic trees were constructed using TreeView (Clustal, 2008; Page, 1996). PThe identification process began with the extraction of genomic DNA from bacterial cells. The 16S rDNA gene fragments were amplified using polymerase chain reaction (PCR). The resulting nucleotide sequences were aligned using Clustal X for multiple sequence alignment. Phylogenetic analysis was then carried out using the neighbor-joining method and visualized with TreeView to facilitate the interpretation of genetic relationships among the bacterial isolates (Suryadi *et al*., 2014).

3. RESULT AND DISCUSSION

3.1 Isolation of Copper-Resistant Bacteria

The isolation and selection process of bacteria grown on LB agar medium containing 3 mM $CuSO_4$ resulted in the identification of five distinct bacterial colony types. Each of these colonies was assigned a specific code: CuFr 1.1, CuFr 1.2, CuFr 1.3, CuFr 2.1, and CuFr 2.2. The designation "CuFr" refers to copper (Cu) isolates from the Tembagapura Freeport mining site (Figure 1).

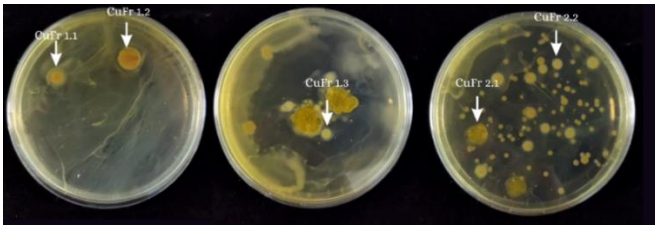

Figure 1. The results of bacterial isolation on medium containing 3 mM $CuSO_4$

3.2 Morphological Characterization of Copper-Resistant Bacterial Colonies

The obtained bacterial isolates were subsequently purified and characterized based on differences in colony morphology. The characterization of isolates included aspects such as color, shape, edge, optical properties, and Gram staining. The results of the morphological characterization of the bacterial isolates are presented in Table 1.

Tabel 1. Characterization Results of Copper-Resistant Bacterial Isolates

No.	Isolat Code	Color	Colony Shape	Margin	Optical Appereance	Gram Strain	Cell Shape
1.	CuFr 1.1	Brown	Round	Smooth	Opaque	Negative	Round
2.	CuFr 1.2	Brown	Round	Smooth	Opaque	Negative	Round
3.	CuFr 1.3	White	Round	Smooth	Opaque	Negative	Round
4.	CuFr 2.1	Green	Irregular	Serrated	Opaque	Negative	Round
5.	CuFr 2.2	White	Round	Smooth	Opaque	Negative	Round

Gram staining was performed to determine the cell shape and the type of bacterial cell wall composition (Irawati, 2019). The results of the Gram staining revealed that all bacterial isolates were classified as Gram-negative with coccoid (round) cell morphology. Gram-negative bacterial cells appeared pink after staining because they are unable to retain the crystal violet dye used as the primary stain. This is due to their thinner cell wall structure. Differences in bacterial cell wall structure affect their resistance to environmental stressors (Rini & Rohmah, 2020). Gram-negative bacteria have a thinner peptidoglycan layer compared to Gram-positive bacteria, which makes Gram-positive bacteria more resistant

to osmotic pressure within the bacterial intracellular fluid and environmental changes. The thickness of the peptidoglycan layer, as a key component of the cell wall, results in a more rigid cell wall that is more difficult to disrupt (Hafsan, 2011; Rini & Rohmah, 2020).

All isolates were identified as Gram-negative bacteria, indicating that all the isolated bacteria possess high resistance to contaminants. Gram-negative bacteria have a different cell wall composition compared to Gram-positive bacteria because they possess an outer membrane, periplasmic space, lipopolysaccharides (LPS), phospholipids, and porins (Irawati, 2021). The outer membrane is unique to Gram-negative bacteria and functions to protect bacterial cells from harmful environments, including chemicals, enzymes, and antibiotics. This outer membrane contains special protein pores called porins, which regulate the flow of molecules into and out of the bacterial cell. The presence of porins enables bacteria to selectively uptake essential compounds such as nutrients while preventing harmful substances from entering the cell (Koentjoro & Prasetyo, 2020).

Lipopolysaccharides (LPS) in Gram-negative bacteria play a role in controlling the activity of endotoxins, which are toxic compounds integrated into the bacterial cell wall and serve as specific antigens according to bacterial type. LPS acts as a molecular signature to stimulate the immune system by triggering immune responses and inducing cytokine production, which can involve inflammation (Munasir, 2016; Purwanto & Astrawinata, 2018). The components of the Gram-negative bacterial cell wall confer important properties and functions in bacterial interactions with their environment. Gram-negative bacteria can adapt to copper-contaminated environments due to their ability to produce specific enzymes that break down pollutants into energy sources to support their growth (Irawati, 2021).

All isolates exhibited colony morphology with a uniform optical appearance described as opaque. Colony color observation revealed that isolates CuFr 1.1 and CuFr 1.2 shared a brown color, CuFr 1.3 and CuFr 2.2 were white, while isolate CuFr 2.1 appeared green. Isolates CuFr 1.1, CuFr 1.2, CuFr 1.3, and CuFr 2.2 displayed round shapes with smooth edges, whereas isolate CuFr 2.1 showed an irregular shape with serrated edges. These morphological characterizations indicate that the bacterial community present in the copper-contaminated soil from the Tembagapura Mining area in Papua is diverse.

3.3 Bacterial Resistance to Copper Testing

The results of the resistance test showed that isolates CuFr 1.1 and CuFr 1.3 were the most resistant strains, as they were able to grow at copper concentrations up to 8 mM, but not at 9 mM. Therefore, both isolates have a minimum inhibitory concentration (MIC) value of 9 mM $CuSO_4$. In comparison, isolates CuFr 1.2, CuFr 2.2, and CuFr 2.1 exhibited MIC values of 7 mM (Table 2). The MIC value serves as an indicator of a bacterium's level of resistance to copper (Suryadi *et al., 2013)*. The isolates obtained from the copper-contaminated soil in the Tembagapura mining site demonstrated high resistance levels, ranging from 7 mM to 9 mM. According to Altimira *et al.* (2012), bacteria are considered highly resistant to copper if they have MIC values greater than 4 mM $CuSO_4$. Given their highest resistance levels, isolates CuFr 1.1 and CuFr 1.3 were selected for further research.

Table 2. Bacterial Resistance to Copper Testing

No.	Isolat Code	MIC Test								
		1mM	2mM	3mM	4mM	5mM	6mM	7mM	8mM	9 mM
1.	CuFr 1.1	√	√	√	√	√	√	√	√	-
2.	CuFr 1.2	√	√	√	√	√	√	-	-	-
3.	CuFr 1.3	√	√	√	√	√	√	√	√	-
4.	CuFr 2.1	√	√	√	√	√	√	-	-	-
5.	CuFr 2.2	√	√	√	√	√	√	-	-	-

The resistance test results of CuFr 1.1 and CuFr 1.3 isolates on media containing 1 mM to 8 mM $CuSO_4$ showed that both bacteria were able to grow well at concentrations between 1 mM and 5 mM. The absence of differences in colony density at these concentrations indicates that copper in this range does not exhibit toxic effects. However, bacterial growth began to decline in terms of colony density at concentrations of 6 mM and 7 mM, and only a few colonies were able to grow at 8 mM (Figures 2 and 3). Copper is an essential trace element, but at elevated concentrations, it becomes toxic. The increasing concentration of $CuSO_4$ led to a decrease in bacterial growth activity. Copper-resistant bacteria possess specific mechanisms that regulate the internal copper concentration. These mechanisms enable the bacteria to allow the necessary amount of copper to enter the cytoplasm while binding the excess copper to membrane-associated fractions, thereby preventing toxicity (Irawati, 2019).

Bacterial resistance to copper involves several key mechanisms, including the action of efflux pumps, copper-binding proteins, and genetic regulation. Efflux pumps are transmembrane proteins that actively transport toxic compounds out of the cell (Afrina *et al*., 2018). A well-known example of this mechanism is the Resistance-Nodulation-Division (RND) system, which utilizes the proton gradient to expel copper ions from the cell. Another example is the P-type ATPase, such as CopA, which uses energy derived from adenosine triphosphate (ATP) to export copper ions from the cytoplasm either to the extracellular space or into intracellular vesicles. Copper-binding proteins function by sequestering copper ions with high affinity, thereby reducing the levels of free copper that could otherwise cause cellular damage. Examples of such proteins include metallothioneins and chaperones. Metallothioneins are rich in cysteine residues, enabling them to bind heavy metals like copper effectively. Chaperones, on the other hand, play a role in binding

cytoplasmic copper and facilitating its transport and homeostatic regulation within the cell. In addition, bacteria possess genetic regulatory mechanisms that modulate copper resistance in response to environmental conditions. One example is CueR in Escherichia coli, a transcriptional regulator that activates the expression of genes associated with copper resistance, such as those encoding efflux pumps and copper-binding proteins, when intracellular copper concentrations rise (Hyre *et al*., 2021).

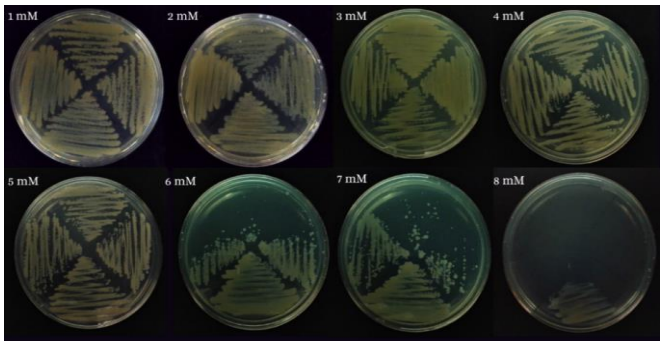

Figure 2. Copper Resistance Test Results for Isolate CuFr 1.1

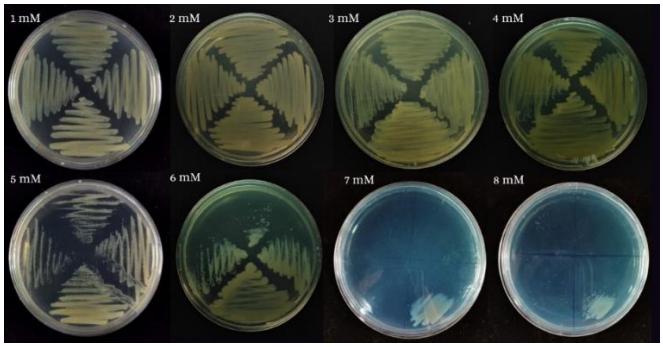

Figure 3. Copper Resistance Test Results for Isolate CuFr 1.3

3.4 Identification of Bacterial Isolates Based on 16S rDNA Gene

The identification results based on the 16S rDNA gene revealed that isolate CuFr 1.1 shared 100% sequence similarity with *Pseudomonas alcaligenes*, while isolate CuFr 1.3 showed 100% similarity with *Pseudomonas chengduensis* (Table 3). Accordingly, the two isolates were identified as *Pseudomonas alcaligenes* CuFr 1.1 and *Pseudomonas chengduensis* CuFr 1.3, respectively. A study conducted by Shukla & Sahoo (1997) demonstrated that *Pseudomonas alcaligenes* is capable of growing in media containing copper concentrations up to 1 mM. *Pseudomonas chengduensis*, on the other hand, was only recently discovered in 2014 (Tao *et al*., 2014) and research on its potential as a copper-resistant bacterium remains very limited.

The 16S rDNA gene-based method is used to determine the similarity of bacterial isolates to previously known bacterial species (Rau *et al*., 2018). The phylogenetic relationship of the isolates can be analyzed through the nitrogen base sequence using the 16S rRNA gene method. Based on the 16S rDNA gene test, isolate CuFr 1.1 exhibited the highest similarity, with a 100% match to *Pseudomonas alcaligenes*. This is evidenced by the dominant appearance of this species name in the similarity percentage calculation table. *Pseudomonas alcaligenes* is characterized as a Gram-negative, rod-shaped bacterium with dimensions ranging from 0.5–1.0 × 0.5–2.6 μm (Thoyib *et al*., 2007).

Tabel 3. Identification Results and Phylogenetic Tree of the 16S rDNA Gene for Isolate CuFr 1.1

Isolat Code	Bacterial Name	Similarity	Accession Number
CuFr 1.1	*Pseudomonas alcaligenes* strain B2 16S ribosomal RNA gene, partial sequence	100%	MH151251.1
CuFr 1.3	*Pseudomonas chengduensis* strain T1624 16S ribosomal RNA gene, partial sequence	100%	CP095766.1

The phylogenetic tree of bacterial isolates CuFr 1.1 and CuFr 1.3 shows that both isolates are closely related to *Pseudomonas alcaligenes* and *Pseudomonas chengduensis* (Figure 4). This relationship was determined through bacterial identification by amplifying the 16S rDNA gene and sequencing the nucleotides to compare the base sequences with those in gene databases. From this identification process, it was confirmed that isolates CuFr 1.1 and CuFr 1.3 share morphological characteristics with the *Pseudomonas* genus, such as being straight or slightly curved motile rods, catalase-positive, and capable of growing well across a wide range of temperatures (Kaligis *et al*., 2020).

The discovery of five copper-resistant bacterial isolates from the Tembagapura mining site in Papua represents a preliminary study that contributes to the existing collection of copper-resistant bacteria. Further research is needed to explore the potential of these isolates as copper biosorbents, thereby advancing the application of conventional biotechnology in the environmental sector. Conventional biotechnology is defined as the process of modifying living organisms by utilizing microbial functions to produce products beneficial to humans through specific processing techniques (Ramadhana *et al*., 2022).

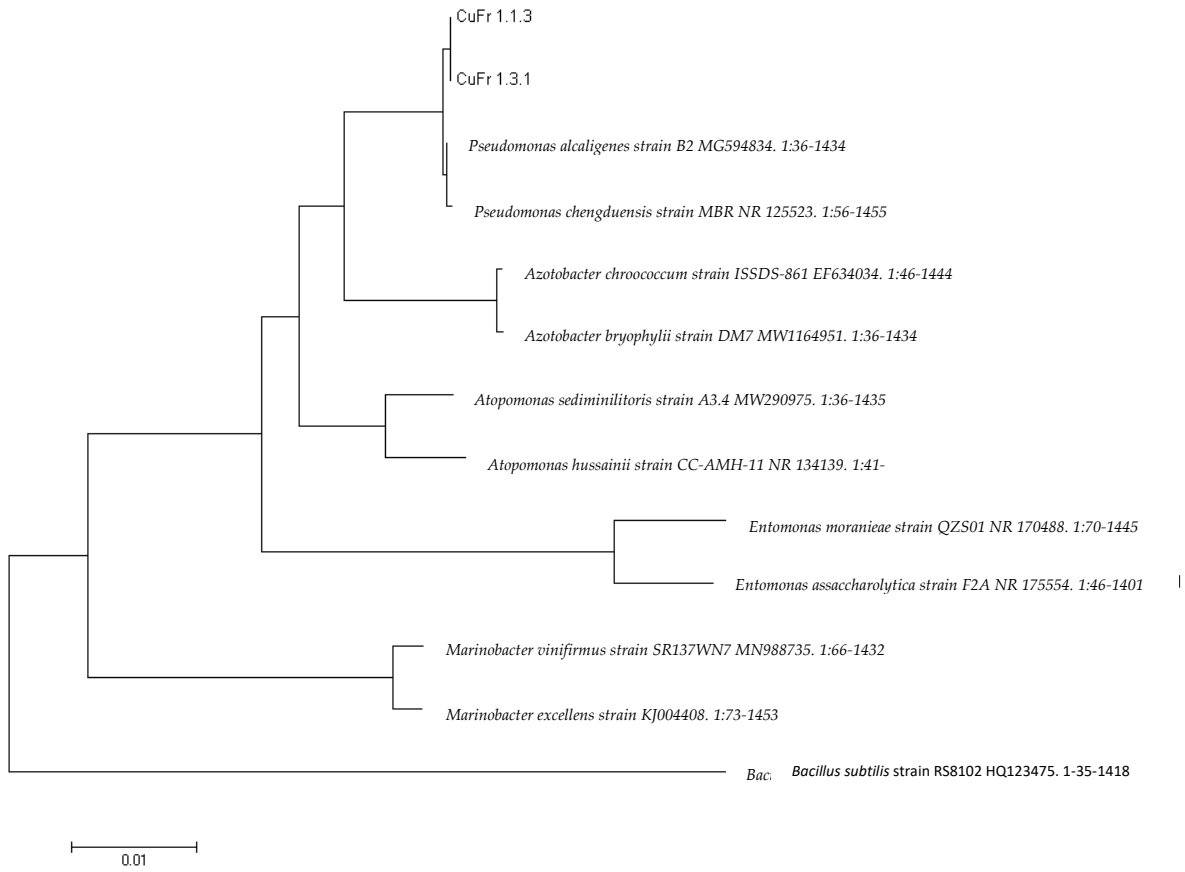

Figure 4. Phylogenetic tree of isolates CuFr 1.1 and CuFr 1.3

4. CONCLUSION

Based on the bacterial isolation results from the mining soil of Tembagapura, Papua, five copper-resistant bacterial isolates were identified: CuFr 1.3, CuFr 2.1, and CuFr 2.2 with a MIC of 7 mM, and CuFr 1.1 and CuFr 1.3 with a MIC of 9 mM. The isolates CuFr 1.1 and CuFr 1.3, which exhibited the highest resistance, were identified as *Pseudomonas alcaligenes* CuFr 1.1 and *Pseudomonas chengduensis* CuFr 1.3, respectively. Physiological and colony morphology characterization revealed that all isolates are Gram-negative, with a turbid optical appearance and round cell shapes. All colony morphologies were round except for isolate CuFr 2.1, which exhibited an irregular, serrated form. Colony colors varied: CuFr 1.1 and CuFr 1.2 were brown, CuFr 1.3 and CuFr 2.2 were white, and CuFr 2.1 was green.

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REFERENCES

Afrina, Nasution, A. I., & Sabila, C. I. (2018). Gambaran Morfologi *Candida albicans* Setelah Terpapar Ekstrak Serai (*Cymbopogon citratus*) pada Berbagai Konsentrasi. Cakradonya Dental Journal, 9(2), 107–115. https://doi.org/10.24815/cdj.v9i2.9748

Ali, N. F. (2017). Analisis Kandungan Logam Berat Timbal (Pb) pada Kerang Simping (*Placuna placenta*) di Perairan Pesisir Utara Kabupaten Gresik. Universitas Airlangga.

Altimira, F., Yáñez, C., Bravo, G., González, M., Rojas, L. A., & Seeger, M. (2012). Characterization of copper-resistant bacteria and bacterial communities from copper-polluted agricultural soils of central Chile. BMC Microbiology, 12(193). https://doi.org/10.1186/1471-2180-12-193

Boky, H., Umboh, J. M. L., & Ratag, B. (2015). Perbedaan Kandungan Merkuri (Hg) Air Sumur Gali Berdasarkan Jarak dari Sumber Pencemar di Wilayah Pertambangan Rakyat Desa Tatelu I. Jikmu, 5(1), 63–70.

Burkhead, J. L., & Collins, J. F. (2022). Nutrition Information Brief-Copper. Advances in Nutrition, 13(2), 681–683. https://doi.org/10.1093/advances/nmab157

Clustal. (2008). *Clustal X (Version 2.0)* [Computer software]. http://www.clustal.org/clustal2/

Darokar, M. P. (2014). In-silico and real-time bioassays based screening of phytomolecules and their derivatives for activity against common drug resistant bacterial pathogens infecting humans. Doctor of Philosophy Dissertation. 226015.

Ettler, V., Konečný, L., Kovarova, L., Mihaljevič, M., Šebek, O.,

Kříbek, B., Majer, V., Veselovský, F., Penížek, V., Vaněk, A., & Nyambe, I. (2014). Surprisingly contrasting metal distribution and fractionation patterns in copper smelter-affected tropical soils in forested and grassland areas (Mufulira, Zambian Copperbelt). The Science of the Total Environment, 117(24), 473–474. https://doi.org/10.1016/j.scitotenv.2013.11.146

Hafsan. (2011). Mikrobiologi Umum (M. K. Mustami (ed.); 1st ed.). Alauddin Pres.

Hyre, A., Casanova-Hampton, K., & Subashchandrabose, S. (2021). Copper Homeostatic Mechanisms and Their Role in the Virulence of *Escherichia coli* and *Salmonella enterica*. EcoSal Plus, 9(2), 1–21. https://doi.org/10.1128/ecosalplus.esp-0014-2020

Irawati, W. (2019). Isolasi dan Karakterisasi Bakteri Resisten Tembaga dari Pantai Timur Surabaya. BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan), 6(2), 95–105. https://doi.org/10.31289/biolink.v6i2.2558

Irawati, W. (2021). Buku Ajar Mikrobiologi. Penerbit Andi Offset

Irawati, W., Ambarita, P. P., Sihombing, D. L., Ruth Advenita, V. E. S., & Marvella, E. B. (2022). Isolation and characterization of indigenous copper resistant bacteria from Yogyakarta tannery factory waste. Jurnal Biologi Tropis, 22(3), 795–802. https://doi.org/10.29303/jbt.v22i3.3621

Irawati, W., Yuwono, T., Soedarsono, J., & Hartiko, H. (2012). Molecular and physiological characterization of copper-resistant bacteria isolated from activated sludge in an industrial wastewater treatment plant in Rungkut-Surabaya, Indonesia. Microbiology Indonesia, 6(3), 107–116. https://doi.org/10.5454/mi.6.3.3

Irawati,W., Tahya, C.Y., Greisnaningsi. (2022). *Pantoea agglomerans*, *Klebsiella pneumoniae*, and *Shigella flexneri* isolated from the Cisadane River as multiresistant bacteria to copper and dyes, Indonesian Journal of Biotechnology, 27(4), 179-186. DOI 10.22146/ijbiotech.66103

Kaligis, C. J., Nangoy, E., & Mambo, C. D. (2020). Uji efek anti bakteri madu hutan dan madu hitam terhadap bakteri *Staphylococcus aureus, Escherichia coli,* dan *Pseudomonas aeruginosa.* E-Biomedik, 8(1), 112–119. https://ejournal.unsrat.ac.id/index.php/ebiomedik/article/view/28704

Kashulina, G. . (2017). Extreme pollution of soils by emissions of the copper–nickel industrial complex in the Kola Peninsula. Eurasian Soil Science, 50, 837–849. https://doi.org/10.1134/S1064229317070031

Koentjoro, M. P., & Prasetyo, E. N. (2020). Dinamika struktur dinding sel bakteri (T. Lestari ed.). Perpustakaan Nasional RI.

Lutfi, S. R., Wignyanto, & Kurniati, E. (2018). Bioremediasi merkuri menggunakan bakteri indigenous dari limbah penambangan emas di Tumpang Pitu, Banyuwangi. Jurnal Teknologi Pertanian, 19(1), 15–24.

Melati, I. (2020). Pusat Penelitian Limnologi LIPI. Prosiding Seminar Biotik, Rahayu 2005, 272–286.

Munasir, Z. (2016). respons imun terhadap infeksi bakteri. Sari Pediatri, *2*(4), 193. https://doi.org/10.14238/sp2.4.2001.193-7

Musztyfaga, E., Kabała, C., Bielińska, A. U., Cuske, M., & Gałka, B. (2014). Soil Pollution with copper, lead and zinc in the surroundings of large copper ore tailings impoundment. Ochrona Srodowiska i Zasobów Naturalnych, 25(4), 45–49. https://doi.org/10.2478/oszn-2014-0027

Page, R. D. M. (1996). TreeView: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences, 12*(4), 357–358. https://doi.org/10.1093/bioinformatics/12.4.357

Purwanto, D. S., & Astrawinata, D. A. W. (2018). Mekanisme kompleks sepsis dan syok septik. Jurnal Biomedik (Jbm), 10(3), 143. https://doi.org/10.35790/jbm.10.3.2018.21979

Puspitasari, D. J., & Khaeruddin. (2016). Kajian bioremediasi pada tanah tercemar pestisida. KOVALEN : Jurnal Riset Kimia, *2*(3), 98–106.

Ramadhana, A., Wijarini, F., & Ilma, S. (2022). The Perception of Biology Education Students on Modern Biotechnological Issues at Borneo University. Borneo Journal of Biology Education, 4(2), 140–146.

Rau, C. H., Yudistira, A., & Simbala, H. E. I. (2018). Isolasi, identifikasi secara molekuler menggunakan Gen 16S rRNA, dan uji aktivitas antibakteri bakteri simbion endofit yang diisolasi dari alga *Halimeda opuntia*. Pharmacon, 7(2), 53–61. https://doi.org/10.35799/pha.7.2018.19509

Rini, C. S., & Rohmah, J. (2020). Buku Ajar Mata Kuliah Bakteriologi Dasar (M. Mushlih (ed.); Pertama). UMSIDA Press.

Rohmayani, V., Wahyuni, H. I., Agustina, S., & Mursidah, L. (2021). Laporan penelitian. 1–53.

Sağlam, A., Yetişsin, F., Demiralay, M., & Terzi, R. (2016). Copper Stress and Responses in Plants. Plant Metal Interaction Emerging Remediation Techniques, 21–40. https://doi.org/10.1016/B978-0-12-803158-2.00002-3.2016

Shukla, S. M., & Sahoo, N. K. (1997). Copper tolerance in *Pseudomonas alcaligenes*. Journal of Applied Microbiology, 82(3), 330–336. https://doi.org/10.1111/j.1365-2672.1997.tb01952.x

Sireesha, I., Subha, R., & Sumithra, S. (2022). Biosorption of copper ions in aqueous solution by carbonized sunflower stem. Rasayan J.Chem, 15(4), 2267-2273

Suryadi, Y., Priyatno, TP., Samudra, IM., Susilowati, DN., Patricia., & Irawati, W. (2013). Karakterisasi dan identifikasi isolat bakteri endofitik penghambat jamur patogen padi. Jurnal Bul Plasma Nutfah, 17(2), 25-32.

Suryadi, Y., Susilowati, D., Lestari, P., Sutoro, S., Manzila, I., Kadir, T., Albani, S., & Artika, I. (2014). Analisis keragaman genetik isolat bakteri *Xanthomonas oryzae* pv. *oryzae* dari Jawa Barat dan Jawa Tengah berdasarkan analisis ARDRA Gen 16SrRNA. Jurnal Fitopatologi Indonesia, 10(2), 53–60. https://doi.org/10.14692/jfi.10.2.53

Tao, Y., Zhou, Y., He, X., Hu, X., & Li, D. (2014). *Pseudomonas chengduensis* sp. nov., isolated from landfill leachate. International Journal of Systematic and Evolutionary Microbiology, 64(PART 1), 95–100. https://doi.org/10.1099/ijs.0.050294-0

Thoyib, H., Setyaningsih, R., & Suratno. (2007). Seleksi dan identifikasi bakteri alkalifilik penghasil xilanase dari Tanah Bukit Krakitan, Bayat, Klaten. Bioteknologi, 4(1), 6–12. https://doi.org/10.13057/biotek/c040102

Torres, E. (2020). Biosorption: A Review of the latest advances. Processes, 8(12), 1-23. https://doi:10.3390/pr8121584

Tumbel, L. K., Wowor, P. M., & Siagian, K. V. (2017). Uji daya hambat minyak kelapa murni (virgin coconut oil) terhadap pertumbuhan bakteri *Enterococcus faecalis*. E-GIGI, 5(1), 1–6. https://doi.org/10.35790/eg.5.1.2017.15535

World Health Organization. (2018). WHO Housing and Health Guidelines. Geneva: World Health Organization.

Zhen, Y., Ge, L., Chen, Q., Xu, J., Duan, Z., Loor, J. J., & Wang, M. (2022). Latent benefits and toxicity risks transmission chain of high dietary copper along the livestock-environment-plant-human health axis and microbial homeostasis: A Review. Journal of Agricultural and Food Chemical, 70(23), 6943–6962. https://doi.org/10.1021/acs.jafc.2c01367

Zolgharnein, H., Lila, M., Azmi, M., Saad, M. Z., Rahim, A., Abd, C., & Mohamed, R. (2007). Detection of plasmids in heavy metal resistance bacteria isolated from the Persian Gulf and enclosed industrial areas. Iranian Journal, 5(4), 232–239.