AL-KAUNIYAH: Jurnal Biologi, 19(2), 2026, 324-332
Website: http://journal.uinjkt.ac.id/index.php/kauniyah
P-ISSN: 1978-3736, E-ISSN: 2502-6720

MOLECULAR CHARACTERISTICS OF THE MERCURY REDUCTASE GENE *Pseudomonas putida* FROM THE LEBONG GOLD MINE

KARAKTERISTIK MOLEKULER GEN MERKURI REDUKTASE *Pseudomonas putida* DARI TAMBANG EMAS LEBONG

Raul Fadillon[1], Rahma Adelia[2], Waris Rahman[3], Gita Panny Nababan[4], Sipriyadi[1,5]*
[1]*Departement of Biology, Universitas Bengkulu, WR Supratman, Kandang Limun, Bengkulu 38122*
[2]*Departement of Medicine, Universitas Bengkulu, WR Supratman, Kandang Limun, Bengkulu 38122*
[3]*Undergraduate Student of Pharmacy, Universitas Bengkulu, WR Supratman, Kandang Limun, Bengkulu 38122*
[4]*Departement of Geophysics, Universitas Bengkulu, WR Supratman, Kandang Limun, Bengkulu 38122*
[5]*Master Study Programme of Biology, Universitas Bengkulu, WR Supratman, Kandang Limun, Bengkulu 38122*
**Corresponding author:* raulfadillon@gmail.com

Submitted: 23 September 2025; Revised: 27 September 2025; Accepted: 19 April 2026

Abstract

Mercury contamination in Lebong Regency has caused serious impacts on public health and environmental quality. Bacteria living in contaminated environments can adapt and develop resistance to mercury through the presence of the merA gene, which plays an important role in converting mercury into a less toxic form. This study aimed to identify and characterize the merA gene in *Pseudomonas putida*. Molecular identification was carried out using 16S rDNA gene analysis and the polymerase chain reaction (PCR) technique. The obtained DNA sequences were further analyzed to determine gene similarity and phylogenetic relationships. The results showed that the bacterial isolate shared 99% homology with *Pseudomonas putida* strain CRSD9. The merA gene was 820 bp in length and encoded a mercury reductase enzyme consisting of 310 amino acids. This gene showed 97.78% homology with previously reported mercury reductase genes and contained two important domains, namely Pyridine Nucleotide-Disulfide Oxidoreductase (Pyr Redox) and *FAD-linked* domains. These findings indicate that *Pseudomonas putida* has strong potential as a bioremediation agent for reducing mercury pollution in the environment.

Keywords: Bioremediation; Mercury; *Pseudomonas putida*

Abstrak

Pencemaran merkuri di Kabupaten Lebong telah berdampak pada kesehatan masyarakat dan lingkungan. Bakteri yang hidup di lingkungan tercemar dapat beradaptasi dan menjadi tahan terhadap merkuri melalui keberadaan gen merA, yang berperan mengubah merkuri menjadi bentuk yang kurang berbahaya. Penelitian ini bertujuan mengidentifikasi dan mengkarakterisasi gen merA pada bakteri Pseudomonas putida. Identifikasi dilakukan menggunakan analisis gen 16S rDNA dan teknik PCR, kemudian urutan DNA dianalisis untuk mengetahui kesamaan gen dan hubungan kekerabatannya. Hasil penelitian menunjukkan bahwa isolat bakteri memiliki tingkat kemiripan 99% dengan Pseudomonas putida strain CRSD9. Gen merA yang ditemukan berukuran 820 bp dan mengode enzim merkuri reduktase yang terdiri atas 310 asam amino. Gen ini memiliki kemiripan 97,78% dengan gen serupa yang telah diketahui sebelumnya serta mengandung dua domain penting, yaitu Pyridine Nucleotide-Disulfide Oxidoreductase (Pyr Redox) dan FAD-linked. Hasil ini menunjukkan bahwa Pseudomonas putida berpotensi dimanfaatkan sebagai agen bioremediasi untuk mengurangi pencemaran merkuri di lingkungan.

***Kata Kunci**: Bioremediasi; Merkuri; Pseudomonas putida*

Permalink/DOI: http://dx.doi.org/10.15408/kauniyah.v19i2.46513

INTRODUCTION

Gold mining is a job done by the indigenous people in Lebong Regency. According to the Badan Pusat Statistik (BPS) Provinsi Bengkulu, gold mining has traditionally been one of the main professions of the Lebong people (Badan Pusat Statistik (BPS) Provinsi Bengkulu, 2023). Most of these traditional gold mines are illegal gold mines. Illegal gold mining is not only a source of livelihood for local residents but also a source of problems for the people in Lebong. Illegal gold mining flourishes have caused health problems and environmental pollution. One of the most massive illegal gold mines is the gold mine in Tambang Sawah Village, Lebong Regency. According to Lestari et al. (2023), gold mining in Tambang Sawah Village has caused a decline in health and environmental quality. This gold mine still uses the amalgamation method using mercury to separate the gold ores. After the amalgamation process, the remaining amalgamation liquid containing mercury concentrations is released into water bodies such as rivers and rice field irrigation.

Mercury is a heavy metal that is highly toxic. Mercury can poison humans; if accumulated or frequently exposed, it can lead to a decrease in health quality and increase the risk of disease. The mercury compounds used in gold ore amalgamation can be in the form of organic and inorganic compounds, but inorganic types of mercury compounds are more commonly used. According to occupational safety regulations, inorganic mercury compounds are safe if used not exceeding the threshold. The threshold for mercury use is 0.05 mg. Mercury compounds are heavy metals that can accumulate in the food chain and settle in the human body. Mercury can survive for 40 days in the body's organs before being naturally excreted. However, if this mercury continues to accumulate, it can lead to health complications. Short-term effects that often appear are irritation of the skin, eyes, and digestive tract. If ingested, it can cause kidney damage (Ministry of Health of the Republic of Indonesia, 2016). While the long-term effects can be more serious, such as causing neurodegenerative diseases (Habibia et al., 2021).

In addition to having a negative impact on health and the environment, mercury that pollutes water and soil is a habitat for bacteria. The bacteria that live there have developed adaptations to be resistant to mercury. Different types of bacteria have been reported to develop mercury-resistant mechanisms. According to Neneng et al. (2020), bacteria of the genus *Pseudomonas* are bacteria that are capable of degrading mercury. This bacterium can be isolated from the wastewater of the gold mine in Pahawang Village, Central Kalimantan. The same thing has also been reported by Sanjaya et al. (2021), who obtained an isolate of mercury-resistant bacteria from the *Mycolibacterium peregninum* species isolated from water in Bantr Karet Village, Bogor. Recently, Setiawan et al. (2023), have obtained an isolate of mercury-resistant bacteria from water and soil samples from Tambang Sawah Village, Lebong Regency. The bacterial isolates have morphological and biochemical characteristics similar to the genus *Pseudomonas*. This bacterial isolate has a reduction capacity at a concentration of 1,000 ppm under *in vitro* conditions.

Mercury-resistant bacteria can reduce mercury in fairly high concentrations because they have the mercury reductase gene. The mercury reductase gene is a single gene or a group of genes responsible for detoxifying mercury through cellular physiological processes. There are various types of mercury reductase genes, and the most common are merA, merB, and merC. According to Nurfitriani et al. (2020), mercury-resistant bacteria containing the mercury reductase gene, especially the merA. It has a high enough mercury reduction capacity so that it is widely developed as a potential bioremediation agent to overcome environmental pollution. Bioremediation is a fairly effective strategy to mitigate the harmful impacts of mercury in Tambang Sawah Village in Lebong Regency. Considering that gold mining is an activity that cannot be separated from the community in Lebong Regency and the lack of alternative materials that can replace mercury in the gold amalgamation process, this study was conducted, which aims to determine the molecular characteristics of the merA in bacteria *P. putida,* which has the potential to be developed as a future bioremediation agent.

MATERIALS AND METHODS

Materials

Mercury-resistant bacterial isolates: A collection of bacterial isolate cultures with isolate code BRM from the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, was isolated from Tambang Sawah Village, Lebong Regency. 63F/1387R 16S rRNA primers, GoTaq Green Master Mix PCR kit, Nutrient Agar (NA), 70% and 96% alcohol, distilled water (aquadest), Eppendorf tubes, GD columns, collection tubes, spirit burners, cotton, wrapping plastic, aluminum foil, Presto™ Mini gDNA Bacteria Kit (Geneaid), lysozyme buffer, Proteinase-K, GB buffer, W1 buffer, elution buffer, nuclease-free water (NFW), agarose, ethidium bromide (EtBr), tissues, gloves, and face masks.

Methods

Identification of Mercury-Resistant Bacteria Using the 16S rDNA Gene

DNA isolation of Gram-negative bacteria began with bacterial cell centrifugation, followed by resuspension of the pellet in GT Buffer and Proteinase-K, and incubation. GB Buffer and ethanol were then added, and the mixture was transferred to a GD Column for centrifugation and washing. Elution was carried out using 100 µL of preheated Elution Buffer. Purified DNA was stored at -20 °C if water was used as the solvent. Amplification of the 16S rRNA gene was performed using a PCR machine with forward primer 63F (5’-CAG GCC TAA CAC ATG CAA GTC-3’) and reverse primer 1378R (5’-GGG CGG WGT GTA CAA GGC-3’), targeting a ~1300 bp fragment. The PCR protocol included pre-denaturation at 94 °C for 5 minutes, denaturation at 94°C for 45 seconds, annealing at 55°C for 1 minute, and elongation at 72 °C for 1 minute and 10 seconds. These three stages were repeated for 30 cycles. This was followed by post-elongation at 72 °C for 7 minutes and cooling at 15 °C for 15 minutes. DNA amplification products were visualized on 1% agarose gel via electrophoresis at 50 V for 50 minutes. The gel was then stained with 0.1% ethidium bromide for 15 minutes and washed with distilled water (ddH$_2$O) for 5 minutes. The agarose gel was visualized using a gel documentation system (gel doc), and the resulting image was saved to a computer (Marchesi et al., 1998).

Molecular Characterisation of Mercury Reductase Gene of *Pseudomonas putida*

The gene encoding mercury reductase was amplified using the forward primer AF (5’-TGTGTCGAAGTTGGCAAGC-3’) and reverse primer AR (5’ ATCCAGCCATCACCGTTTTG-3’). PCR reactions were carried out using a PCR machine. Each reaction consisted of 25 µL buffer, 1 µL of each primer (10 µM), 1 µL Taq polymerase, 3 µL template DNA, and nuclease-free water to a final volume of 50 µL. The PCR program included initial denaturation (94 °C for 5 minutes), denaturation (94 °C for 2 minutes), annealing (60 °C for 1 minute), elongation (72 °C for 1 minute), and final elongation (72 °C for 10 minutes). DNA amplification was carried out for 34 cycles. Sequencing of the mercury reductase gene was analyzed using the NCBI server (Cabral et al., 2016).

Sequencing of 16S rDNA and Mercury Reductase Genes of *Pseudomonas putida*

PCR products were sent to First Base, Malaysia, via PT. Genetika Science Indonesia for nucleotide sequencing. The sequence results were processed using BioEdit software, and low-quality chromatograms were trimmed to obtain high-quality contiguous sequences (contigs). Sequences were aligned using ClustalW in the MEGA 11 software. The processed and edited DNA sequences were compared to the GenBank database using the Basic Local Alignment Search Tool for nucleotides (BLAST) to obtain homologous sequences. A phylogenetic tree was constructed based on these homologous sequences using the MEGA 11 software with the bootstrap method (1,000 replicates). The tree was built using the Neighbor-Joining method (Tamura et al., 2013). The data on the characterization of mercury reductase genes from mercury-resistant bacterial isolates are presented in visual form and described qualitatively. Nucleotide sequences were analyzed using MEGA 11 bioinformatics software to determine gene homology. DNA sequences were also analyzed using the NCBI Basic Local Alignment Search Tool (Toha, 2016).

RESULTS

Identification of Mercury-Resistant Bacteria Isolate Using 16S Ribosomal DNA Gene

Identification of mercury-resistant bacterial isolates was carried out using the 16S ribosomal DNA gene. Amplification of the mercury-resistant bacterial isolates BRM was performed using the PCR technique with primers 63F and 1387R. The PCR products were then analyzed by electrophoresis using 1% agarose gel. The PCR amplification of the 16S ribosomal DNA gene of the mercury-resistant bacterial isolates produced a DNA fragment of approximately 1,300 bp. The visualization of the amplified 16S ribosomal DNA can be seen in Figure 1.

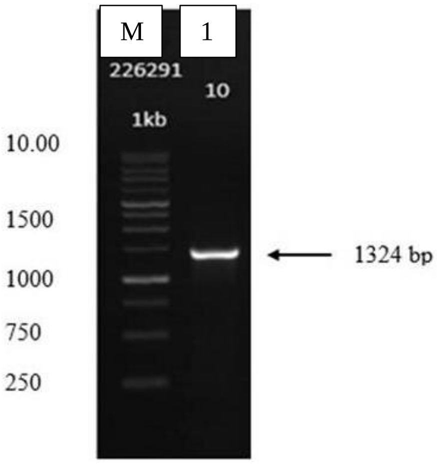

Figure 1. PCR amplification of the 16S rRNA gene of mercury-resistant bacterial isolates showed a target DNA band of 1,324 bp. M = 1 kb DNA Ladder (Thermo Scientific), 1 PCR product of the 16S rRNA gene from the mercury-resistant bacterial isolate

The nucleotide sequence data of the 16S rRNA gene of the mercury-resistant bacteria isolate BRM were compared with the 16S rRNA gene sequences of other bacteria available in GenBank using the BLASTn program from NCBI. The sequence analysis of the 16S rRNA gene showed that a minimum of 97% nucleotide sequence similarity is required to determine that the species are the same. The homology of the mercury-resistant bacterial isolate based on NCBI is presented in Table 1.

Table 1. Homology of the mercury-resistant bacteria isolate based on BLASTn from the NCBI

Isolate code	Reference strains	Total nucleotide	Similarity (%)	E-value	Accession number (GeneBank)
BRM	*P. plecoglukosida* strain ER29	719	98.81	0.00	MT124554
	P. putida strain CSRD9	680	99.81	0.00	MT641244
	P. monteilii strain JM10	773	99.81	0.00	MT605299
	P. viridiflava strain Aceter 10B	717	99.81	0.00	MT386206

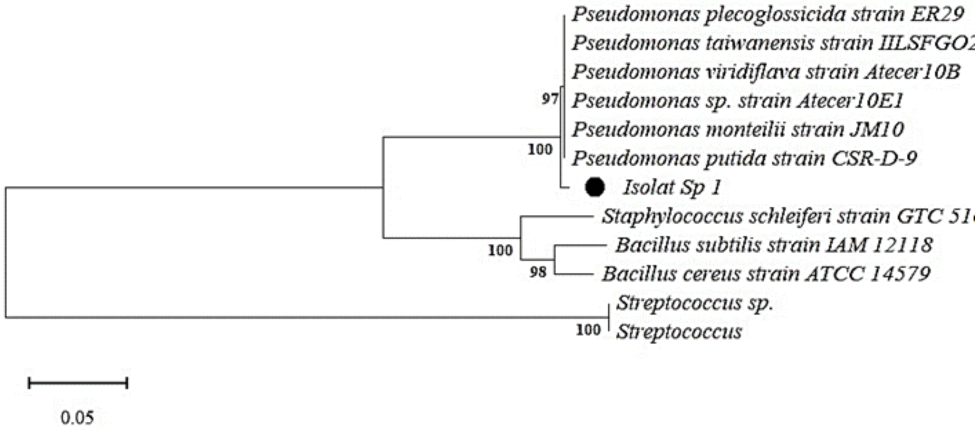

Figure 2. Phylogenetic tree construction of bacterial isolate BRM based on the 16S rRNA gene sequence using the neighbor-joining method with 1,000 bootstrap replications

The phylogenetic tree construction based on the 16S rRNA gene was carried out using the neighbor-joining method with 1,000 bootstrap replications. The phylogenetic tree analysis aimed to determine the relationship among bacterial isolates with 16S rRNA gene sequences. The reference bacterial strains used for phylogenetic tree construction were those most closely related according to GenBank. The result of the phylogenetic tree construction of the mercury-resistant bacteria isolate BRM is shown in Figure 2.

Characteristics of the merA gene of *Pseudomonas putida*

The presence of mercury resistance genes was detected based on the sequence of the *merA* gene. Detection of the merA gene was carried out through PCR amplification. This study successfully amplified the merA gene in the *P. putida* isolate, producing an amplicon of 820 bp (Figure 3).

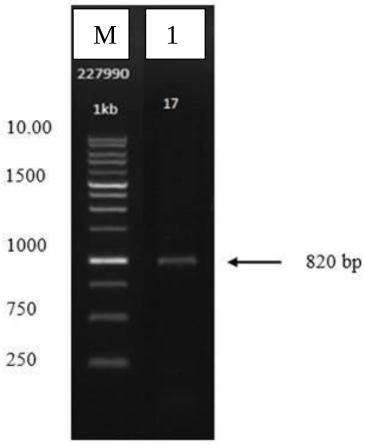

Figure 3. PCR amplification of the merA gene (±820 bp) from *Pseudomonas putida*, detected using agarose gel electrophoresis. Marker = 1 kb DNA ladder (Thermo Scientific), 1= PCR product of the merA gene from the *Pseudomonas putida*

The presence of a band corresponding to the 820 bp size of the merA gene confirms that the *P. putida* strain has the potential to reduce mercury. The homology of the merA gene based on BLASTn is presented in Table 2.

Protein analysis of the merA gene was conducted using the three-dimensional structural model of the partial merA protein and evaluation through a Ramachandran plot. The three-dimensional model provides an overview of the spatial arrangement and folding pattern of the protein, while the Ramachandran plot was used to assess the stereochemical quality of the predicted structure by analyzing the distribution of dihedral angles of the amino acid residues. The results of the structural modeling and Ramachandran plot analysis of the partial merA protein are presented in Figure 4.

The alignment between the partial amino acid sequence of merA *P. putida* and the full-length reference amino acid sequence of merA from *P. aeruginosa* indicates that the predicted domain of partial merA *P. putida* is located in the *Pyr redox 2* and *FAD-linked* domain (Figure 5). In order to characterize the gene, the domains of the merA protein complete sequence were analyzed using the protein classification website InterPro (Figure 5). Reference sequences were obtained from the RCSB Protein Data Bank (RCSB PDB). For merA, the reference confirmed the presence of two domains, namely *Pyr redox 2* domain and *FAD-linked* domain. The protein sequence of merA from *P. putida* had linear results but only included the full domain of the *FAD-linked* domain and some part of the *Pyr 2* redox domain, although it only covered part of the *FAD-linked* domain.

Table 2. BlastX species homology of the merA gene in *Pseudomonas putida*

Species	Homology	Query covered (%)	E-value	Similarity	Accession number
P. putida	*Pseudomonas* mercury (II) reductase	100%	(0.0)	97.78%	WP_320521114
	Pseudomonas mercury ion reductase	100%	(0.0)	97.78%	CAB65946
	Pseudomonas FAD-dependent oxyreductase	100%	(0.0)	97.78%	EKT4497506

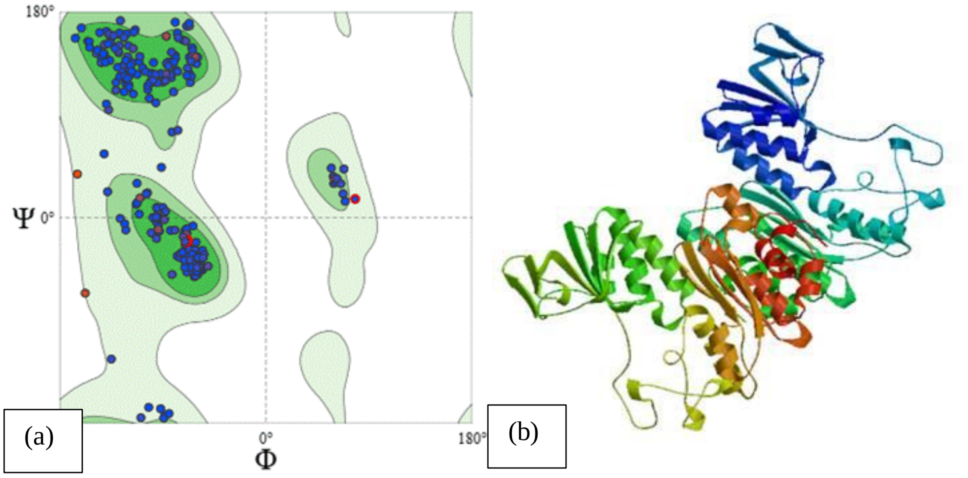

Figure 4. Molecular characteristics of the merA gene from *Pseudomonas putida*. Ramachandran plot (a), the majority of residues are located within the allowed conformational regions, indicating that the protein structural model is stable and stereochemically valid. Protein structure of the characteristic mercury reductase gene (b). The 3D structure of this protein shows a complex folding composed of a combination of α-helices, β-sheets, and loops that are interconnected to form a stable and well-organized three-dimensional conformation

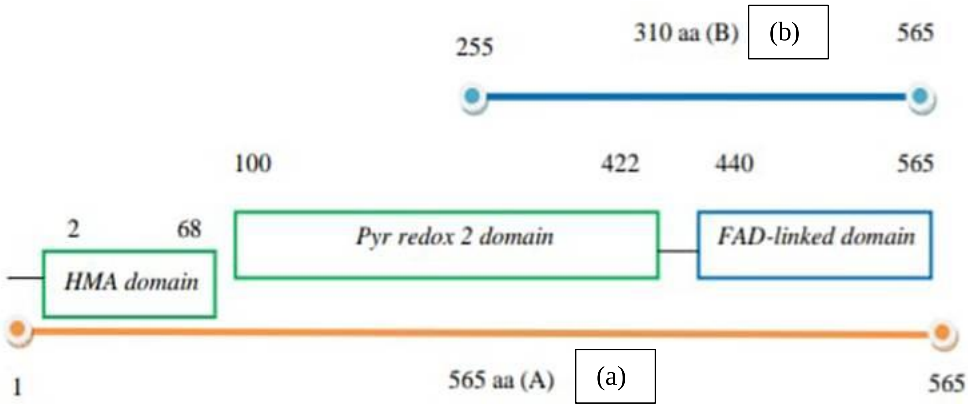

Figure 5. Predicted domains of merA *Pseudomonas putida* aligned with reference complete sequences (RCSB PBD, 565 amino acids) (a) and partial sequence of merA from *Pseudomonas putida* contains 310 amino acids (b)

DISCUSSION

The visualization of the 16S ribosomal DNA gene, shown in Figure 1, reveals a clear and distinct band at approximately 1,324 bp. According to Mora et al. (2025), the 16S ribosomal DNA gene typically ranges from 1,300 to 1,550 bp in size. The DNA visualization results indicate successful amplification of the 16S ribosomal DNA gene via PCR, which can be used for sequencing in subsequent procedures. Further research by Badaruddin and Yusuf (2011) states that the 16S rRNA gene can be used to detect the presence of mercury-resistant bacteria. In addition, a BLAST analysis was conducted to determine the degree of similarity between the 16S rRNA sequence of the mercury-resistant bacterial isolate and the GenBank sequences, as shown in Table 1. Table 1 indicates that the mercury-resistant bacterial isolate is closely related to the genus *Pseudomonas*. The BLAST analysis of the 16S ribosomal DNA gene from the mercury-resistant bacterial isolate against the NCBI database revealed a 99.81% similarity with *P. putida*, *P. montelii*, and *P. viridiflava*, which showed the highest identification percentage and query coverage. According to Newell et al. (2013), percent identification on the NCBI site shows the nucleotide similarity between the sample and the reference sequence. Additionally, Newell et al. (2013), emphasized that query coverage is the similarity between the sample's nucleotide length and the sequence, which strengthens the bacterial identification down to the strain level.

After performing the BLASTn test on the 16S ribosomal DNA sequence of the mercury-resistant bacterial isolate, a phylogenetic tree reconstruction was carried out. The phylogenetic tree of the 16S ribosomal DNA gene from the mercury-resistant bacterial isolate, shown in Figure 3, illustrates the close relationship between the 16S rRNA sequence of the isolate and the sequences of other species used for comparison. The closest phylogenetic relationship of the mercury-resistant bacterial isolate is depicted by examining the nearest branch with the highest bootstrap value. According to the phylogenetic reconstruction, the mercury-resistant bacterial isolate is most closely related to *P. putida* strain CSRD9 (MT641244). The bootstrap percentage for the phylogenetic tree reconstruction is 100%, indicating the highest confidence in the relationship of the mercury-resistant bacterium. The bootstrap value in the phylogenetic reconstruction using the Neighbor Joining method, as explained by Tamura et al. (2013), measures the confidence or stability of the tree branches. This value is obtained through repeated resampling of the data, determining how consistent each branch appears in the repetitions. A high bootstrap value (≥70%) indicates strong data support for a particular branch, while a low value suggests instability. Thus, the bootstrap value helps validate the structure of the phylogenetic tree and enhances the reliability of bacterial classification interpretation. Bacteria classified as the genus *Pseudomonas* have morphological and physiological characteristics. The genus *Pseudomonas* is a rod-shaped Gram-negative bacterium that does not form spores, is motile with polar flagella, and shows aerobic metabolism with positive oxidase and catalase tests (Li et al., 2025). Species of this genus are very diverse; examples are *P. aeruginosa*, *P. viridiflava*, and *P. fluorescens*.

One species known for mercury bioremediation capabilities is *P. putida* (Tasleem et al., 2023). *P. putida* bacteria belong to the group of Gram-negative bacteria that belong to the class *Gammaproteobacteri*a and the family *Pseudomonadaceae*. The genus *Pseudomonas* itself is a genetically diverse group of bacteria and includes hundreds of species that are widely distributed in various environments such as soil, water, and plants. *P. putida* has high genetic diversity and plays an important role in a variety of ecological processes, including the degradation of organic compounds and environmental bioremediation (Udaondo et al., 2024). A distinguishing feature of *P. putida* is its ability to degrade organic pollutants, making it an important agent in bioremediation. *P. putida* plays a key role in mercury bioremediation through its ability to reduce toxic mercury ions (Hg^{2+}) to a less harmful elemental form (Hg^0) using the mercury reductase enzyme (merA). Some species are also capable of breaking down toxic organomercury compounds such as methylmercury with the organomercury lyase enzyme (merB). The merA gene is generally located on plasmids, enabling resistance transfer between bacteria (Christakis et al., 2021).

The visualization of the merA gene, shown in Figure 3, reveals a distinct band of approximately 820 bp. Based on the PCR amplification results, the merA gene was successfully detected in the mercury-resistant bacterial isolate. According to Sotero-Martins et al. (2008), the merA gene has a length of 820 bp and can serve as a molecular marker. This gene can be used for the identification of mercury-resistant bacterial strains. The BLAST analysis of the merA gene from the mercury-resistant bacterial isolate shows a similarity of 98.89% with *P. putida*. According to Purkan et al. (2016), several gram-negative bacteria known to be resistant to mercury include *Pseudomonas* sp., *Flavobacterium* sp., *Thiobacillus* sp., *Escherichia coli*, *Acinetobacter* sp., *Enterobacteriaceae*, *Xanthomonas* sp., *Aeromonas* sp., *Erwinia* sp., *Rhodococcus* sp., *Oerskovia* sp., *Staphylococcus* sp., *Micrococcus roseus*, and *Citreobacterium* sp. On the other hand, gram-positive bacteria resistant to mercury include *Bacillus* sp. and *Staphylococcus aureus*.

Based on the results of the analysis, the merA It is known to encode as many as 310 amino acids (Figure 5). The orange line represents the amino acid sequence of the reference merA gene with a full length of 565 amino acids, containing three main domains, the HMA domain at residues 2–68, which plays a role in metal ion binding; the *Pyr redox 2* domain at residues 100–422, which functions in oxidoreductase activity; and the *FAD-linked* domain at residues 440–565, indicating the involvement of the FAD cofactor in catalytic activity. Meanwhile, the blue line represents the deduced amino acid sequence of the merA gene, which only covers a portion of the protein, namely residues 255–565 (310 amino acids), thus including only part of the *Pyr redox* 2 domain and the entire *FAD-linked* domain. The difference in sequence length indicates that the deduced merA gene does not represent the full-length protein domains but still retains the key regions associated with oxidoreductase function and FAD binding.

CONCLUSION

Analysis of the 16S rRNA gene showed that the mercury-resistant bacterial isolate had a sequence similarity of 99% with *Pseudomonas putida* strain CSRD9. The mercury reductase gene (merA) was most closely related to *Pseudomonas putida* strain MRSN 365855 and was successfully amplified, producing a DNA fragment 820 base pairs long. Sequence analysis results show that the merA gene has 100% sequence coverage and a 97.78% similarity to the gene encoding mercury (II) reductase enzyme. This gene is responsible for 310 amino acids that form the mercury reductase enzyme and belongs to the Flavin Adenine Dinucleotide (FAD) oxidoreductase protein family. The merA gene has two main conserved domains, namely the include the *Pyr redox 2* domain at residues 100–422, which functions in oxidoreductase activity; and the *FAD-linked* domain at residues 440–565, which play an important role in the catalytic activity of the enzyme to reduce mercury ions (Hg^{2+}) into a less toxic form. The results of this study indicate that the merA gene has great potential in mercury detoxification mechanisms, and further research is needed to assess its effectiveness and application in mercury bioremediation.

ACKNOWLEDMENTS

This study was supported by the Ministry of Education, Culture, Research, and Technology of Indonesia (Kemdikbud Ristek) under Grant No. 4362/E2/KM.01.00/2023 through the 2023 Student Creativity Program in Exact Sciences (PKM-RE), as well as the University QS.100 University Malaya Program Implementation Arrangement. The authors gratefully acknowledge the Microbiology and Biotechnology Laboratory, Department of Biology, Bengkulu University, for providing the mercury-resistant bacterial isolate used in this study. The authors also extend their sincere appreciation to colleagues and the PKM-RE 2023 alumni for their valuable contributions and constructive support throughout the research. Special thanks are extended to Redo Setiwan for his substantial assistance with the wet laboratory experiments.

REFERENCES

Badaruddin, F., & Yusuf, I. (2011). Isolasi dan identifikasi bakteri resisten merkuri dari muara Sungai Sario yang dapat digunakan untuk detoksifikasi limbah merkuri. *Jurnal Ilmiah Sains*, *11*(2), 283-288.

Badan Pusat Statistik (BPS) Provinsi Bengkulu. (2023). Jumlah perusahaan dan tenaga kerja menurut klasifikasi industri pada industri besar dan sedang. (2024, November 19). Retrieved from https://bengkulu.bps.go.id/id/statistics-table/1/MTc1OSMx/jumlah-perusahaan-dan-tenaga-kerja-menurut-klasifikasi-industri-pada-industri-besar-dan-sedang--2023.html. Accessed: 19 November.

Cabral, L., Yu, R. Q., Crane, S., Giovanella, P., Barkay, T., & Camargo, F. A. O. (2016). Methylmercury degradation by *Pseudomonas putida* V1. *Ecotoxicology and Environmental Safety*, *130*(2016), 37-42. doi: 10.1016/j.ecoenv.2016.03.036.

Christakis, C. A., Barkay, T., & Boyd, E. S. (2021). Expanded diversity and phylogeny of mer genes broadens mercury resistance paradigms and reveals an origin for merA among thermophilic Archaea. *Frontiers in Microbiology*, *12*(2024), 1-20. doi: 10.3389/fmicb.2021.682605.

Habibia, A. M., Ferry, Y., Hen, S., Karimah, P. A. P., & Wia, B. R. (2021). Penyakit Parkinson akibat merkuri pada pekerja penambangan emas skala kecil. *CoMPHI Journal: Community Medicine and Public Health of Indonesia Journal*, *1*(3), 170-176.

Lestari, D. F., Fatimatuzzahra, F., Febrianti, E., Wulansari, S. S., & Atmaja, V. Y. (2023). Edukasi bahaya merkuri bagi kesehatan wanita di kawasan tambang emas Kabupaten Lebong Provinsi Bengkulu. *Jurnal Pengabdian UNDIKMA*, *4*(2), 355-361.

Li, H., Qian, Z., Yan, Y., & Wang, H. (2025). Isolation, identification, and drug Sensitivity test of *Pseudomonas aeruginosa* from Cynomolgus monkey (*Macaca fascicularis*). *Veterinary Sciences*, *12*(7), 636. doi: 10.3390/vetsci12070636.

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J., & Wade, W. G. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Applied and Environmental Microbiology*, *64*(2), 795-799.

Ministry of Health of the Republic of Indonesia. (2016). *Regulation of the Minister of Health of the Republic of Indonesia number 57 of 2016: National action plan for the control of health impacts due to mercury exposure*. Jakarta: Ministry of Health of the Republic of Indonesia.

Mora, C. C., Rojas, C. J. A., Rosales, V. M. C., Urban, M. J. L., Delgado, E., Medrano, R. H., … Jáquez, D. R. (2025). Identification of microorganisms at different times in a bioleaching process for the recovery of gold and silver from minerals in oxide form. *Heliyon*, *11*(2), 1-10. doi: 10.1016/J.HELIYON.2025.E41878.

Neneng, L., Ardianoor, A., Usup, H. L. D., Adam, C., Zakaria, Z., Ghazella, A., … Alvianita, V. (2020). Potensi *Chlorella* sp. dan *Pseudomonas* sp. dari areal tambang emas sebagai mikroorganisme potensial pereduksi merkuri. *Jurnal Ilmu Lingkungan*, *18*(3), 617-625. doi: 10.14710/jil.18.3.617-625.

Newell, P. D., Fricker, A. D., Roco, C. A., Chandrangsu, P., & Merkel, S. M. (2013). A small-group activity introducing the use and interpretation of BLAST. *Journal of Microbiology & Biology Education*, *14*(2), 238-243. doi: 10.1128/jmbe.v14i2.637.

Nurfitriani, S., Arisoesilaningsih, E., Nuraini, Y., & Handayanto, E. (2020). Bioaccumulation of mercury by bacteria isolated from small-scale gold mining tailings in Lombok, Indonesia. *Journal of Ecological Engineering*, *21*(6), 127-136. doi: 10.12911/22998993/123247.

Purkan, P., Nurmalyya, S., & Hadi, S. (2016). Resistance level of *Pseudomonas stutzeri* against mercury and its ability in the production of mercury reductase enzyme. *Molekul*, *11*(2), 230-238. doi: 10.20884/1.jm.2016.11.2.256.

Sanjaya, W. T. A., Khoirunnisa, N. S., Ismiani, S., Hazra, F., & Santosa, D. A. (2021). Isolation and characterization of mercury-resistant microbes from gold mine area in Mount Pongkor, Bogor District, Indonesia. *Biodiversitas*, *22*(7), 2656-2666. doi: 10.13057/BIODIV/D220714.

Setiawan, R., Wibowo, R. H., Sipriyadi, S., Mashudi, M., & Wulandari, G. D. (2023). Isolation and characterization of mercury-resistant bacteria from gold mining in Lebong, Bengkulu. *IOP Conference Series: Earth and Environmental Science*, *1271*(1), 1-9. doi: 10.1088/1755-1315/1271/1/012075.

Sotero-Martins, A., Silva De Jesus, M., Lacerda, M., Moreira, J. C., Luzia, A., Filgueiras, L., … Escola, C. (2008). A conservative region of the mercuric reductase gene (*merA*) as a molecular marker of bacterial mercury resistance. *Brazilian Journal of Microbiology*, *39*(2008), 307-310.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12),2725-2729. doi: 10.1093/molbev/mst197.

Tasleem, M., El-Sayed, A.-A. A. A., Hussein, W. M., & Alrehaily, A. (2023). *Pseudomonas putida* metallothionein: Structural analysis and implications of sustainable heavy metal detoxification in Madinah. *Toxics*, *11*(10), 864. doi: 10.3390/toxics11100864.

Toha, A. H. A., Widodo, N., Hakim, L., & Sumitro, S. B. (2016). *Panduan dasar analisis data genetik untuk publikasi*. Papua: Marine Biodiversity of Raja Ampat Island.

Udaondo, Z., Ramos, J. L., & Abram, K. (2024). Unraveling the genomic diversity of the *Pseudomonas putida* group: Exploring taxonomy, core pangenome, and antibiotic Resistance mechanisms. *FEMS Microbiology Reviews*, *48*(6). doi: 10.1093/femsre/fuae025.