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Metallothionein Protein Modeling from *Pseudomonas aeruginosa* PAO1 as A Metal Biosorber Candidate

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Abstract. *Metallothionein is a protein that is well known to play a role in metal metabolism in bacterial cells. Metallothionein is a multifunctional protein that has the potential to be used as a metal adsorbing agent. Pseudomonas aeruginosa is a ubiquitous gram-negative and rapid-growth bacterium. In addition, the complete genome of Pseudomonas aeruginosa has been largely known. Pseudomonas aeruginosa PAO1 is a strain of Pseudomonas aeruginosa that the complete genome of this strain is easily accessible in NCBI. These features make Pseudomonas aeruginosa PAO1 become a common model in bacterial studies. This research aimed to find and model the putative metallothionein of Pseudomonas aeruginosa PAO1. This research was carried out by bioinformatic and protein homology methods. Based on the results, the putative metallothionein of Pseudomonas aeruginosa PAO1 was found in the bacterial genome at base sequence of 2355918 to 2356157. The putative metallothionein-encoding gene of Pseudomonas aeruginosa PAO1 has a size of 240 bp. The translation result of the gene showed that the putative metallothionein of Pseudomonas aeruginosa PAO1 has 79 amino acids. The modeling result showed the 3D structure of the putative metallothionein of Pseudomonas aeruginosa PAO1 is similar to the metallothionein 3D structure of Pseudomonas fluorescens Q2-87. The 3D structure of the putative metallothionein of Pseudomonas aeruginosa PAO1 was dominated by turn and coil, but contained 1 α-helix structure and 2 β-sheet structures. Based on protein analysis, it was found that the putative metallothionein of Pseudomonas aeruginosa PAO1 has 1 metal-binding cluster with 10 amino acids and the most important amino acid residue is Cysteine . Even though, there was 1 Histidine amino acid residue on the metal-binding cluster.*

Keywords: *metallothionein, metal biosorber, protein modelling, Pseudomonas aeruginosa PAO1*

Citation
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INTRODUCTION

Biosorption is a metal absorption method using biological agents. Biosorption is often used as a method to absorb heavy metals from wastewater. This method has several advantages over other methods such as low cost, free of side products, and fast processing time (Giese et al., 2020). In addition, biosorption also does not produce chemical sludge which is a big problem in chemical precipitation methods when absorbing heavy metals (Choi

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& Yun, 2004). Biosorption can be carried out using bacteria, yeast, fungi, algae, and plants (Ali Redha, 2020; Chen et al., 2021; Martinez et al., 2023; Ord et al., 2023; Therdkiattikul et al., 2020). In the case of bacteria, several discovered metal-absorbing bacteria are Pseudomonas alchaliphila, *Pseudomonas putida* S4, *Azotobacter* S8, *Bacillus subtilis*, *Pseudomonas aeruginosa* strain FZ-2, and *Klebsiela oxytoca* (El-Naggar et al., 2020; Imron et al., 2021; Kurniawan et al., 2019; Lin et al., 2021; Nokman et al., 2019). There are two mechanisms of biosorption in general, namely extracellular and intracellular (Pham et al., 2022). The extracellular biosorption mechanism is carried out by complexation, ion exchange, and physical adsorption. The intracellular biosorption mechanism is carried out by bioaccumulation involving metal-binding proteins (Naik & Dubey, 2013).

In addition to a method used in remediating metal-polluted environments and waste treatment, biosorption is also used in biomining, especially bioleaching. Bioleaching is a metal extraction technology from low-quality ores and mineral concentrates using microorganisms (Bosecker, 1997). Bioleaching can be used to collect metals from metal sulfide minerals. Microorganisms having extraction ability come from two groups of microorganisms, namely bacteria and archaea (Dopson & Okibe, 2023). Several bacteria, such as *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Leptospirillum ferriphilum*, and *Acidithiobacillus thiooxidans*, have been already known to have ability to absorb metals (Amar et al., 2021; Li et al., 2021; Loa et al., 2021; Sundramurthy et al., 2020). In current developments, bioleaching has been developed as a technology for extracting metals from electronic waste (Baniasadi et al., 2019).

One of the metal-binding proteins that has been studied and has many roles in metabolism is metallothionein (Sharma et al., 2021). Metallothionein is a metal-binding protein found in almost all living things. Metallothionein plays a role in the regulation of metals in living cells. Studies show that metallothionein is a protein that plays a role in the resistance of living things to heavy metals. SmtA is a metallothionein protein that causes *Synechococcus* PCC7942 to have resistance to Zn2+, Cd2+, Cu2+, and Hg2+ metal ions because it can bind these metals (Blindauer, 2011). MymT is a metallothionein protein from *Mycobacterium tuberculosis* that can bind Cu+ ions and makes *Mycobacterium tuberculosis* resistant to Cu (Gold et al., 2008). Metallothionein is not only found in *Synechococcus* PCC7942 and *Mycobacterium tuberculosis* but also found in *Bacillus cereus*, *Pseudomonas fluorescens* Q2-87, *Staphylococcus aureus*, *Enterobacter cloacae*, and *Klebsiella* sp. (Chudobova et al., 2014; Habjanic et al., 2020; Haq et al., 1999; Murthy et al., 2011). The amino acid sequence of metallothionein contains a lot of cysteines (Chatterjee et al., 2020). In metallothionein, cysteine is an amino acid residue that binds metal ions through the metal-binding cluster. However, the metal-binding clusters of some metallothioneins involve Histidine as a metal-binding amino acid residue. Because of its ability to bind metals, metallothionein has the potential to be employed as a biosorbent in the absorbing process of heavy metals from the environment, waste treatment, and the bioleaching process.

Pseudomonas aeruginosa is a ubiquitous gram-negative and rapid-growth bacterium (LaBauve & Wargo, 2012). *P. aeruginosa* can grow easily in many environments with a wide variety of nutrients. Because of these features, *P. aeruginosa* has been a model in bacterial study for a long time. *P. aeruginosa* PAO1 is a strain of *P. aeruginosa* that has

become a model in bacterial genetic and protein study because the complete genome that has been known and published in The National Center for Biotechnology Information (NCBI) (Stover et al., 2000). *P. aeruginosa* PAO1's genome containing as many as 6.3 million base pairs could be easily accessed from the database. Despite the complete genome of *P. aeruginosa* PAO1, metallothionein and metallothionein-encoding gene of this bacterium have not been studied yet specifically and comprehensively. This fact remains the gap that could be explored to learn the potency of metallothionein of *P. aeruginosa* PAO1 as a metal biosorber.

In this research, The 3D structure of metallothionein of *P. aeruginosa* PAO1 is constructed to explore the potency of metallothionein as a biosorbent in the metal uptake process. This research studied the position of metallothionein-encoding genes in the genome of *P. aeruginosa* PAO1, 3D structure based on protein structure homology, and its metal-binding cluster. The position of metallothionein-encoding gene informs us of the outside gene area. This data will be useful to develop the specific or universal primer to amplify the metallothionein-encoding gene. The 3D structure and its metal-binding cluster inform us how the metallothionein binds the metal and what residues are involved in the metal-binding process, especially the metallothionein of *P. aeruginosa* PAO1. This information will be useful in developing the engineered-metal biosorbent that has better absorption ability than the wild type that can be applied in heavy metal absorption, metal waste treatment, and metal extraction.

MATERIALS AND METHODS

The research was conducted from June until July 2023 at Universitas Sultan Ageng Tirtayasa, Banten, Indonesia.

Finding The Metallothionein-Encoding Gene of *Pseudomonas aeruginosa* PAO1

The metallothionein-encoding gene of *P. aeruginosa* PAO1 was obtained by using the alignment method. In each gene, there are conserved residues that will always be the same even though the gene is in different organisms (Dale & Park, 2004). If the alignment process is conducted to some genes, these conserved residues will be placed in a parallel state (Choudhuri, 2014). Thus, a specific gene in a bacterium could be found by using the same gene of other bacteria that its sequence has been discovered. In this case, the metallothionein-encoding gene of *P. aeruginosa* N6P6, bmtA, was aligned to the genome of *P. aeruginosa* PAO1 through the BLASTN program in The National Center of Biotechnology Information (NCBI) website. The bmtA is gene-coding metallothionein in *P. aeruginosa* N6P6 that causes this bacterium to have resistance to Pb, Cd, Hg, Cr, and Zn. (Kumari & Das, 2019). Using bmtA as the template in the BLASTN program with *P. aeruginosa* PAO1 as the target object and selecting the *P. aeruginosa* PAO1 complete genome (access code AE004091.2) for further quest, the putative metallothionein-encoding gene of the *P. aeruginosa* PAO1 could be found (Ismail, 2022). The sequence of bmtA is shown in Figure 1. The putative metallothionein-encoding gene of *P. aeruginosa* PAO1 obtained from that alignment was used as a metallothionein protein template on the protein structure modeling.

Protein Modeling

The metallothionein of *P. aeruginosa* PAO1 was built by using SWISS-MODEL online software. The gene sequence obtained from the BLASTN was then translated into

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a protein sequence. The resulting protein sequence was used as the target sequence and its 3D structure was modeled by using SWISS-MODEL with the steps as follows:

```
          10         20         30         40         50         60         70
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
1   ATGAACGATCAACGCTGCGCGTGTACGCACTGTTCCTGCACTGTGGATGCCAATGCCTTGCAGCGCGATG

          80         90        100        110        120        130        140
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
71  GCAAGGCCTATTGCTGCGAGGCCTGCGCCAGCGGCCACCGCAAGGGTGAGCCATGCCGGATGCAGGACTG

         150        160        170        180        190        200        210
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
141 CCATTGTGGTGAAAAGCCGGGCGAGAGCGCGGTGGACAATGCGCTGGATGAAACCTTTCCAGCGAGTGAG


    ....|....
211 CCGATCTCG
```

Figure 1. The sequence of bmtA

Input data

The target sequence was provided as a FASTA file.

Template Research

The template is a structure used as a reference to determine the structure of the target sequence. SWISS-MODEL aligns the target sequence with structures in SWISS-MODEL Template Library (SMTL) (Waterhouse et al., 2018). The properties resulting from this target-template alignment were then used to find the proper template. SWISS-MODEL employs two database search methods in the target-template alignment: BLAST and Hhblits (Biasini et al., 2014). These methods give good alignment results eventhough in low identity sequence level.

Template Selection

Template search generated several templates with a set of properties based on target-template alignment. The proper template can be determined according to the GMQE score (Waterhouse et al., 2018). Global Model Quality Estimate (GMQE) is the score calculated by combining the properties from the target-template alignment. GMQE score expresses the accuracy and reliability of the template. GMQE score ranges between 0 to 1 with 1 expressing the highest accuracy and 0 expressing the lowest accuracy (Biasini et al., 2014).

Model Building

The 3D structure of the target sequence was modeled based on the selected template. The modeling process began bytransferring the conserved atom coordinates that were defined by target-template alignment (Waterhouse et al., 2018). Then, amino acids corresponding to insertion or deletion and non-conserved amino acid sidechains were modeled in. The process was performed by ProMod 3, based on OpenStructure computational structural biology framework (Biasini et al., 2013).

Model Estimation

The 3D structure suggested by the model-building process were examined and validated by several parameters such as GMQE, QMEAN Z-score, and MolProbity. Similar

to the template selection, the GMQE score expresses the accuracy and reliability of the model. The model has good accuracy if GMQE score is close to 1, while the GMQE score close to 0 meaning that the model has a poor accuracy. . QMEAN Z-score shows how the model when compared with the experimental structure of similar size (degree of nativeness). A higher QMEAN Z-score relates to more favorable models and a lower QMEAN Z-score relates to more unfavorable models (Benkert et al., 2011). MolProbity is a structure-validation web service that examines the model quality at both global and local levels for both protein and nucleic acid. The validation is based on the power and sensitivity provided by optimized hydrogen placement, all-atom contact analysis, covalent geometry, and torsion-angel criteria. MolProbity provides a result table that shows validation and the goal criteria. In addition, the MolProbity result table is complemented by the traffic light-color coding to help the users determine whether the result is a good or a bad value. The quality of the structure is expressed by the MolProbity score (Chen et al., 2010; Williams et al., 2018).

DNA and Protein Analysis

The metallothionein-encoding gene of *P. aeruginosa* PAO1 was analyzed to know both its size and position in the genome. These informations are very important for developing specific primers, cloning the gene, and engineering the gene and the protein (Choudhuri, 2014). The analysis was carried out by alignment method through the BLASTN program and BioEdit software. According to the step of finding the metallothionein-encoding gene of *P. aeruginosa* PAO1, the size and the position of the metallothionein-encoding gene in the *P. aeruginosa* PAO1 genome could be found precisely by selecting the *P. aeruginosa* PAO1 genome with access code of AE004091.2. The genome of *P. aeruginosa* PAO1 was downloaded and analyzed further by using BioEdit software.

The metallothionein structure generated from protein modeling was analyzed for its primary structure and metal-binding cluster. This information is important to engineer the protein for both overexpression of the protein as a biosorbent and enhancement of protein-absorption ability. The metal-binding cluster is a group of amino acids that play a role in metal binding. The primary structure was analyzed by BioEdit software while the metal-binding cluster was analyzed by using Visual Molecular Dynamics (VMD) and LigPlus software. The analysis of the metal-binding cluster used the comparison between the metallothionein of *P. aeruginosa* Q2-87 as a reference and the metallothionein of *P. aeruginosa* PAO1 as a target object. Analysis of the metallothionein of *Pseudomonas fluorescens* Q2-87 using LigPlot software gives the results of the amino acids involved in the metal-binding cluster of the metallothionein. Comparing the structure of metallothionein of *P. aeruginosa* PAO1 with the structure of metallothionein of *P. luorescens* Q2-87, the amino acids involved in the metal-binding cluster of metallothionein of *P. aeruginosa* PAO1 could be determined.

RESULTS AND DISCUSSION

Finding The Metallothionein-Encoding Gene and DNA Analysis

Alignment result between the bmtA and all genes in the genome of *P. aeruginosa* PAO1 showed that there are five variants of *P. aeruginosa* PAO1 genome which had genes similar to the bmtA. These similar genes may be the metallothionein-encoding gene of *P. aeruginosa* PAO1. These five variants of *P.*

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aeruginosa PAO1 genome are shown in Table 1. The result of the alignment also showed that the genes from the five variants of the *P. aeruginosa* PAO1 genome that are similar to bmtA had the same percent identity and query cover, namely 71.12% and 78%, respectively. This result indicated that the genes similar to the bmtA are the same gene and may be metallothionein-encoding genes (Barghouthi, 2011; Ismail, 2022; Thareau et al., 2003). By selecting the genome of *P. aeruginosa* PAO1 with access code AE004091.2 for further analysis, the putative metallothionein-encoding gene of *P. aeruginosa* PAO1 has been found in base 2355918 until base 2356157 with 240 bp in size. The sequence of putative metallothionein-encoding gene of *P. aeruginosa* PAO1 is shown in Figure 2.

Table 1. Amino acid residues of the metal-binding cluster of the putative metallothionein of *Pseudomonas aeruginosa* PAO1 and Metallothionein of *P. fluorescens* Q2-87

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc Len	Accession
Pseudomonas aeruginosa PAO1 chromose, complete genom	*Pseudomonas aeruginosa* PAO1	83.3	121	78%	9e-16	71.12%	6217899	CP053028.1
Pseudomonas aeruginosa PAO1. H2O genome	*Pseudomonas aeruginosa* PAO1.H2O	83.3	121	78%	9e-16	71.12%	6264404	CP008749.1
Pseudomonas aeruginosa PAO1-V13 genome	*Pseudomonas aeruginosa* PAO1-V13	83.3	121	78%	9e-16	71.12%	6265484	CP006832.1
Pseudomonas aeruginosa PAO1-V2 genome	*Pseudomonas aeruginosa* PAO1-V2	83.3	121	78%	9e-16	71.12%	6265484	CP006831.1
Pseudomonas aeruginosa PAO1 genome	*Pseudomonas aeruginosa* PAO1	83.3	121	78%	9e-16	71.12%	6264404	AE004091.2

```
          10        20        30        40        50        60        70
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
1   ATGAACAGCGAAACCTGTGCCTGTCCCAAATGCACCTGCCAGCCCGGCGCCGATGCCGTCGAACGCGACG

          80        90        100       110       120       130       140
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
71  GCCAGCACTATTGCTGCGCGGCCTGCGCCAGCGGCCACCCCCAGGGCGAGCCGTGTCGCGACGCCGATTG

          150       160       170       180       190       200       210
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
141 CCCCTGTGGCGGCACCACCCGGCCCCAGGTGGCGGAGGATCGCCAGCTGGACGACGCGTTGAAGGAGACC

          220       230       240
    ....|....|....|....|....|....|
211 TTTCCCGCCAGCGACCCGATCTCGCCCTGA
```

Figure 2. The sequence of putative metallothionein-encoding gene of *Pseudomonas aeruginosa* PAO1

Metallothionein Modeling

The amino acid sequence of Pseudomonas aeruginosa PAO1 putative metallothionein was gained by translating the putative metallothionein-encoding gene of *P. aeruginosa* PAO1. The amino acid sequence of *P. aeruginosa* PAO1 putative metallothionein is shown in Figure 3. The putative metallothionein of *P. aeruginosa* PAO1 has 79 amino acids with a mass of 8.3 kDa. This is consistent with the general features of metallothionein which generally has ~ 60 amino acids with a molecular mass of ~ 6 kDa (Gutiérrez et al., 2019).

The template search results showed the top 5 templates suggested by SWISS-MODEL for modeling the 3D structure of the putative metallothionein of *P. aeruginosa* PAO1 (Table 2) and metallothionein of *P. aeruginosa* Q2-87 were template with GMQE of 0.73 and coverage of 0.99 (Biasini et al., 2014). The 3D structure of the putative metallothionein of *P. aeruginosa* PAO1 is shown in Figure 4. The 3D structure modeled in Figure 4 has a GMQE of 0.59, QMEAN Z-score of -4.2, the MolProbity score, and Ramachandran favoured of 84th percentile and 84.21%, respectively. GMQE of 0.59 means the 3D model of putative metallothionein of *P. aeruginosa* PAO1 has a medium accuracy as it is closer to 1 than 0. QMEAN Z-score of -4.2 means the 3D model of putative metallothionein of *P. aeruginosa* PAO1 is unfavourable in terms of nativeness. This may be due to the experimental metallothionein structure of *Pseudomonas* genera is still limited in the database as seen in Table 2. MolProbity is a way to validate a 3D structure model considering all phenomena in the structure including the φ/ψ plot, which is called a Ramachandran plot. MolProbity score is a combined single indicator of model quality (Chen et al., 2010; Williams et al., 2018). MolProbity results of the 3D structure of the putative metallothionein of *P. aeruginosa* PAO1 are shown in Table 3. MolProbity score of this structure is 84th percentile which means the 3D structure of the putative metallothionein of *P. aeruginosa* PAO1 has a medium quality. In summary, all parameters used to examine and validate the 3D structure of the putative metallothionein of P*P. aeruginosa* PAO1 show that this 3D structure model has a medium quality. The 3D structure also showed that the putative metallothionein of *P. aeruginosa* PAO1 has one α-helix structure, two β-sheet structures, some turns, and coils.

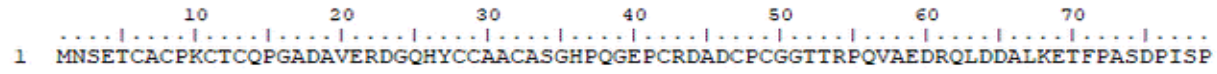

Figure 3. The amino acid sequence of *Pseudomonas aeruginosa* PAO1 putative metallothionein

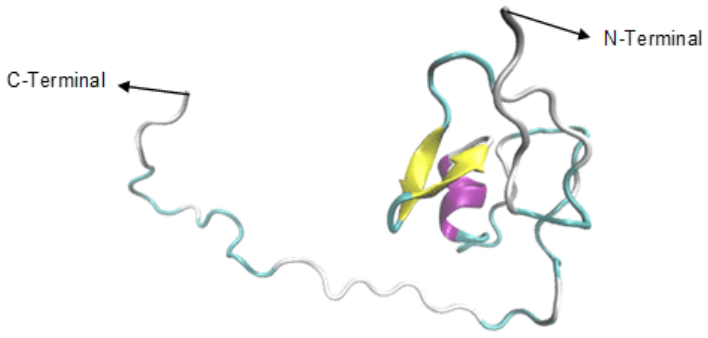

Figure 4. The 3D structure of the putative metallothionein of *Pseudomonas aeruginosa* PAO1

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Table 2. Top 5 Templates suggested by SWISS-MODEL for modeling the 3D structure of the putative metallothionein of *Pseudomonas aeruginosa* PAO1

Template Name	Coverage	GMQE	Identity	Method	Oligo State	Ligands
6grv.1. A Metallothionein Cadmium(II) form of full-length metallothionein from Pseudomonas fluorescens Q2-87 (PflQ2 MT)	0.99	0.73	48.72	NMR	monomer	4 x CD
6gw8.1. A metallothionein Zn(II) form of shortened metallothionein from Pseudomonas fluorescens Q2-87 (residues 1-52)	0.63	0.41	44.00	NMR	monomer	3 x Zn
6gv7.1. A metallothionein Cadmium(II) form of A44H mutant of shortened metallothionein from Pseudomonas fluorescens Q2-87 (residues 1-52)	0.63	0.40	44.00	NMR	monomer	4 x CD
6gv6.1. A metallothionein Cadmium(II) form of shortened metallothionein from Pseudomonas fluorescens Q2-87 (residues: 1-52)	0.63	0.40	44.00	NMR	monomer	4 x CD
6gv7.1. A metallothionein Cadmium(II) form of A44H mutant of shortened metallothionein from Pseudomonas fluorescens Q2-87 (residues 1-52)	0.61	0.38	47.92	NMR	monomer	4 x CD

Table 3. MolProbity Results

Category	Result	Goal
MolProbity Score	1.84 (84th percentile)	100th percentile is the best among structures of comparable resolution; 0th percentile is the worst
Clash Score	3.76 (96th percentile)	100th percentile is the best among structures of comparable resolution; 0th percentile is the worst
Ramachandran Favoured	84.21%	>98%
Ramachandran Outliers	2.63%	<0.05%
Rotamer Outliers	0.00%	<0.3%
C-Beta Deviation	1	0
Bad Bonds	1 / 574 (0.17%)	0%
Bad Angle	4 / 783 (0.51%)	<0.1%

Metallothionein Analysis

The results of the analysis of the primary structure showed that the putative metallothionein of *P. aeruginosa* PAO1 contains 10 Cys residues with a percentage of 12.66%. Cys is one of the high-percentage amino acids in the putative metallothionein of *P. aeruginosa* PAO1. The other high-percentage amino acids are Ala at 12.66%, Pro at 11.39%, and Asp at 10.13%. The number of Cys residues in metallothionein is at least around 8-10 residues. Generally, we cannot say a protein is a metallothionein if the percentage of Cys contained in the protein is smaller than the other amino acids (Blindauer, 2009; Gutiérrez et al., 2019; Hidalgo et al., 2009). This general feature confirms that the putative metallothionein of *P. aeruginosa* PAO1 is a metallothionein.

Searching for metallothionein conserved amino acid residues from 6 bacteria having high similarity with *P. aeruginosa* PAO1 showed that there are 32 conserved amino acid residues in metallothionein (Figure 5). In these metallothionein-conserved residues, there is a metal-binding cluster that contains 10 amino acids (Table 4). The metal-binding cluster is a cluster of amino acids responsible for metal absorption in bacteria (Blindauer, 2009, 2011; Gutiérrez et al., 2011, 2019; Hidalgo et al., 2009). Information on the metal-binding cluster is very important because metal-binding cluster is an active site of metallothioneins which would be the main part of the engineering process of metallothioneins to enhance the protein-absorption ability.

Cysteine is the predominant amino acid in the metal-binding cluster (Blindauer, 2009; Chatterjee et al., 2020; Gutiérrez et al., 2019; Hidalgo et al., 2009). However, the metal-binding cluster of this putative metallothionein has histidine in the sequence number 36. Histidine is a residue that is rarely found in vertebrate metallothionein (Gutiérrez et al., 2011; Hidalgo et al., 2009), but in bacterial metallothionein, histidine becomes a characteristic residue and is involved in the metal-binding cluster together with cysteine (Blindauer, 2009, 2011). In addition, histidine also plays a role in stabilizing protein folding and metal group charges in bacterial metallothionein (Blindauer et al., 2007). The first four cysteines of the metal-binding cluster of the metallothionein of *P. fluorescens* Q2-87 and the putative metallothionein of *P. aeruginosa* PAO1 have different sequence numbers (Table 4). This is caused by metallothionein of Pseudomonas fluorescens Q2-87 not preceded by methionine. However, the alignment process can prove that all the amino acid residues in the metal-binding clusters are conserved (Figure 5). Amino acid residues that play a role in the metal-binding cluster of the putative metallothionein of *P. aeruginosa* PAO1 are shown in Figure 6.

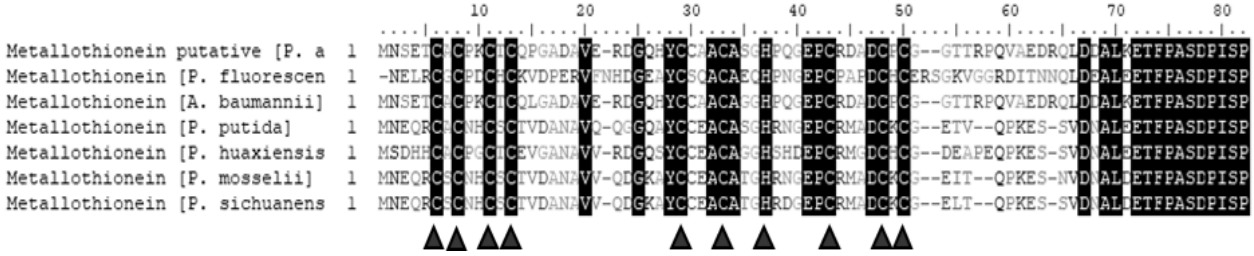

Figure 5. Conserved amino acid residues of metallothionieins : metal-binding cluster amino acids

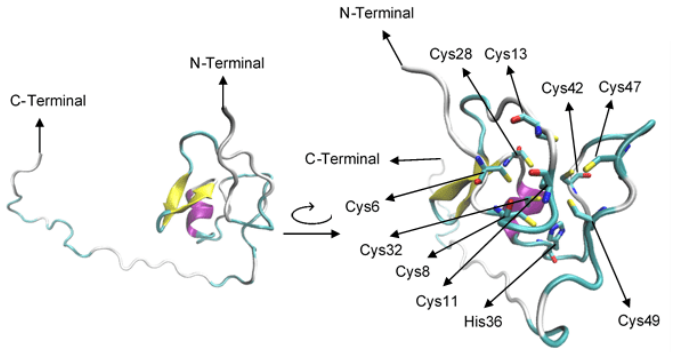

Figure 6. Amino acid residues of the metal-binding cluster of the putative metallothionein of *Pseudomonas aeruginosa* PAO1

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Table 4. Amino acid residues of the metal-binding cluster of the putative metallothionein of *Pseudomonas aeruginosa* PAO1 and Metallothionein of P. fluorescens Q2-87

Metallothionein of *P. fluorescens* Q2-87 (template)	**Putative Metallothionein of *P. aeruginosa* PAO1**
Cys5, Cys7, Cys10, Cys12, Cys28, Cys32, Cys42, Cys47, Cys49, His36	Cys6, Cys8, Cys11, Cys13, Cys28, Cys32, Cys42, Cys47, Cys49, His36

CONCLUSION

The putative metallothionein of *Pseudomonas aeruginosa* PAO1 has been successfully modeled. Based on the modeling results, the putative metallothionein of *P. aeruginosa* PAO1 has a close 3D structure to the metallothionein of *P. aeruginosa* Q2-87. The 3D structure model of putative metallothionein of *P. aeruginosa* PAO1 has the MolProbity score of 84th percentile which means the quality of this 3D structure model is medium. The putative metallothionein of *P. aeruginosa* PAO1 has one metal-binding cluster containing ten amino acids. The metal-binding cluster of metallothionein *P. aeruginosa* PAO1 contains 9 cysteines and 1 histidine. These amino acids are the main part of the engineering process to support the function of the metallothionein as a metal biosorber, especially the heavy metals, bioremediation of heavy metal polluted environments, metal waste treatment, and development of a metal-extracting agent. *P. aeruginosa* is a bacterium that can be found in soil. That fact indicates that we could use some soil bacteria having metallothionein-encoding genes as a source of metallothionein as a metal biosorber.

AUTHOR CONTRIBUTION

F.I. designed the research and supervised all the process, A.S. ran the modeling process, analyzed the data, and wrote the manuscript. S.A. collected and analyzed the data of the gene and wrote the manuscript.

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CONFLICT OF INTEREST

There is no conflict of interest.

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