Research Article

Complete Genome Sequencing of Polar Arthrobacter sp. PAMC25284, Copper Tolerance Potential Unraveled with Genomic Analysis

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1.Introduction

Glacier habitats have rich and diverse microbial communities with unique adaptive characteristics. Among such cold inhabitants, Actinobacteria with high GC content are considered to be the most common [1]. The phylum Actinobacteria is made up of phylogenetically diverse organisms that have been studied for their ability to cause diseases in plants and animals, produce antimicrobial compounds and antitumor agents, and degrade recalcitrant molecules in soil environments [2]. Within Actinobacteria, members of the genus Arthrobacter are notable because they are among the most frequently found in soil environments. Their widespread distribution is due to their dietary versatility and tolerance to environmental challenges. Compared to mesophilic Arthrobacter isolates, Antarctic Arthrobacter strains showed genome content scaling as an adaptation alteration, exhibiting fewer protein-coding sequences and a lower number of transcription and carbohydrate metabolism-associated genes [1]. Ubiquitous organisms are assumed to have key roles in the biogeochemistry of heavy metals due to their fundamental features as bioconverters [3]. As a result,
studying the responses of microbes to metals is of scientific interest and may be useful in developing biotechnological solutions for the recovery and purification of important and/or harmful metals in the environment.

Copper is a metal ion that has been shown to be hazardous to bacteria and other organisms. Excess copper, regardless of its valence state, binds to a wide range of biomolecules, including proteins, lipids, and nucleic acids [4]. However, unlike other poisonous metals, like silver and lead, copper is also an important trace nutrient. Bacteria developed strict copper homeostatic control systems involving copper binding and transport, as well as copper-mediated gene regulation. The copper resistance is encoded by the cop genes (copA, copB, copC, copD, copY, and copZ) in Cupriavidus metallidurans CH34, P. aeruginosa PAO1, and E. hirae and by the pco genes (pcoA, pcoB, pcoC, and pcoD) in Escherichia coli [5, 6]. CopA and CopB are copper-transporting ATPases, while CopY is a copper-responsive repressor, and CopZ is a chaperone that intracellular copper routing [6]. The copA gene encoding a multi-copper oxidase (pcoA gene in E. coli) is one of the main genetic determinants involved in Cu resistance in Gram-negative bacteria. In E. hirae, this copper can participate in the metalation of copperoenzymes in some rare cases. Copper defense by multi-copper oxidase has been reported in various bacteria, including Campylobacter jejuni, Myxococcus xanthus, Rhodobacter capsulatus, Salmonella enterica, Staphylococcus aureus, and Xanthomonas camppestris, in addition to E. coli [7]. In nature, multi-copper oxidase functionality varies depending on the source organism and the surroundings. Laccases (EC 1.10.3.2) and a broad family of copper oxidases, such as ascorbate oxidases (EC 1.10.3.3), ceruloplasmin (EC 1.16.3.1), bilirubin oxidase (EC 1.10.3.4), and metallo-oxidases Fet3p (EC 1.16.3.1.3), are all included in multi-copper oxidase [8]. In bacteria, they play important roles in spore coat resistance [9], melanin production [10], morphogenesis [11], metal oxidation [12], and denitrification [13]; in fungi, pigment formation [14], lignin degradation [15], dissimilatory nitrite reduction [16], and virulence [17]; in yeasts, iron uptake [18]; in insects, cuticle tanning [19]; in plants, lignin biosynthesis and ascorbate metabolism [20]; in mammals, iron metabolism [21].

In this study, we performed comprehensive genome sequencing on Arthrobacter sp. PAMC25284, a psychrotolerant bacterium originally isolated from seawater collected from the South Shetland Islands, Barton Peninsula, Antarctica. Various genes involved with copper resistance are highlighted herein. To our knowledge, this is the first study to provide genetic and phenotypic insight into Arthrobacter sp. PAMC25284 derived from Antarctica seawater and its potential role in copper resistance.

2. Materials and Methods

2.1. Taxonomic Identification. The Arthrobacter sp. PAMC25284 was isolated from the seawater of the South Shetland Islands, Barton Peninsula (62°13.536′ S; 58°47.054′ W) using 0.1X RA agar (MB cell Ltd., Seoul, Korea), and it was acquired at the environmental temperature of 20°C. The bacterial sample for DNA analysis was done at 15°C temperature using pure R2A agar media. The DNA from strain PAMC25284 was extracted using a QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA, USA). Genome quality and concentration were determined by a spectrophotometer (Biochrome, Libra S35PC, UK) and detected by agarose gel electrophoresis to evaluate its quality.

2.2. Complete Genome Sequencing and Annotation. Genome sequencing was performed using PacBio sequo single-molecule real-time (SMRT) sequencing technology (Pacific Biosciences, Menlo Park, CA, USA). SMRTbell library inserts (20kb) were sequenced using SMRT cells. Raw sequence data were generated from 77,075 reads and 821,081,934bp that were assembled de novo using the hierarchical genome assembly process (HGAP) protocol [22] and HGAP4 assembly using SMRT analysis software (ver. 2.3; Pacific Biosciences, https://github.com/PacificBiosciences/SMRT-Analysis). The complete genome sequence was deposited in the GenBank database under the GenBank accession number NZ_CP080382.1 (Bio project number PRJNA748195).

The PAMC25284 genome was annotated using the rapid annotation subsystem technology (RAST) server [23]. The predicted gene sequences were translated and searched in the National Center for Biotechnology Information (NCBI) nonredundant database, the Cluster of Orthologous Groups (COG) from the eggno g v.4.5.1 database [24], and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. A circular map of the PAMC25284 genome was prepared using the CGViewBETA comparison tool [25].

2.3. Genotypic Analysis of Arthrobacter sp. PAMC25284. The phylogenetic analysis of the Arthrobacter sp. PAMC25284 16s rRNA gene sequence and the sequences of the type strains of the species within the family Micrococcaceae was performed using MEGAX software [26], based on the alignment of the sequence with ClustalW [27]. The distances were calculated using Kimura’s two-parameter model [28], and the phylogenetic tree was inferred using maximum likelihood [29] neighbor joining [30] analysis. For phylogenetic tree construction, complete genome sequences of the 16s RNA and sequences of related type strains were obtained from the EzBioCloud database (http://www.ezbiocloud.net/) [31]. The average nucleotide identity (ANI) values between the genome sequence of strain PAMC25284 and the type strains of the closest related species were estimated using the ANI calculator in the EzBioCloud. The G+C mol.% content of DNA was determined from the complete sequence.

For protein phylogenetic tree construction, the secondary data were used to identify type I copper center protein and its variants. The multi-copper oxidase domain containing the protein sequence of the strain PAMC25284 and type I copper center protein of the related strains were obtained from the UniPort (https://www.uniprot.org/) [32] and NCBI database (https://www.ncbi.nlm.nih.gov/) [33], respectively. The sequences were aligned by MUSCLE.
and a phylogenetic tree was inferred using maximum likelihood and neighbor joining analysis. The multiple sequence alignment of the related proteins and then identification of the conserved region were performed using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) [36]. Signal IP 5.0 [37] (neural networks and Markov models) and TMHMM 2.0 Server [38] were used to predict the subcellular localization of strain PAMC25284 multi-copper oxidase domain-containing proteins.

2.4. Prediction of Cu-Specific Transporters, Chaperones, and Cuproproteins of *Arthrobacter* sp. PAMCC25284. Cuproproteins, chaperons, and copper-specific transporters of a given organism were predicted using previous literature, protein-protein blast (blastp) search [39], and the highest homology sequences were determined. The sequences were retrieved from the RAST database and BLAST was done against the amino acid sequences to obtain the highest homology sequences.

### 3. Results and Discussion

3.1. Complete Genome Profile of *Arthrobacter* sp. PAMC25284. The complete genome of *Arthrobacter* sp. PAMC25284 is comprised of a circular chromosome of 3,883,680 bp with a GC content of 65.6 percent, as shown in Table 1. On the chromosome, 3,588 genes were predicted, with 3,150 protein-encoding genes functionally assigned and the remaining genes predicted as hypothetical proteins. We annotated 366 pseudogenes, 19 rRNA genes, and 50 tRNA genes distributed throughout the genome. Of the predicted genes, 2,860 (90.80%) were classified into 20 functional Clusters of Orthologous Groups (COG) categories, whereas the remaining 290 (9.20%) genes were unclassified. The most numerous COG categories were genes with S genes with unknown function (581 genes), E (289 genes), K (263 genes), and G (223 genes) (Figure 1). Many of these genes are related to amino acid transport, carbohydrate transport, and energy production/conversion. The metabolic flexibility of this strain was discovered through phenotypic assessment of carbon utilization profiles. Furthermore, significantly fewer coding sequences (CDSs) were allocated to the COG categories of transcription [K] and carbohydrate transport and metabolism [G] out of the total CDSs discovered in the genome. In four Antarctic *Arthrobacter* isolates, fewer CDSs, decreased metabolic flexibility, and a significant drop in CDS related to transcription, carbohydrate transport, and metabolism suggest genome content scaling [2].

With further gene subsystem clustering analysis, *Arthrobacter* sp. PAMC25284 with SEED viewer of RAST database showed functional genes with the presence of a total of 279 (26% of the strain’s genome) subsystems [40]. The top five subsystems belonged to carbohydrate metabolism (347); amino acids and derivatives (306); protein metabolism (163); cofactors, vitamins, prosthetic groups, and pigments (140); and nucleosides and nucleotides (83). Additionally, functions related to membrane transport (46); stress response (36); resistance to antibiotics and toxic compounds (31); the metabolism of aromatic compounds (16) were also identified. Collectively, these analyses of cold-adapted *Arthrobacter* sp. PAMC25284 suggest the presence of several genome-enabled metabolic and catabolic processes, which might play a significant role in the colonization and its survival in such psychrophilic environments. Similar findings have been reported in psychrophilic *Cryobacterium* species [41], where specific genes in the categories like carbohydrates, cofactors, vitamins, prosthetic groups, pigments, and ABC transporters in membrane transport were predominant.

### 3.2. Phylogenomic Analysis Based on 16S rRNA and Multi-Copper Oxidase Domains. A phylogenetic tree was constructed based on 16S rRNA sequences that are related to the genus *Arthrobacter* strains, which include strain PAMC25284 (Figure 2). The *Arthrobacter* sp. PAMC25284 shared the same clade with *A. oryzae* KV-651T with 62% bootstrap support and *A. humicola* KV-653T with 95% bootstrap support. According to the EzBioCloud database, five *Arthrobacter* species (*A. oryzae* KV-651T, *A. humicola* KV-653T, *A. pascens* DSM20545T, *A. globiformis* NBRC12137T, and *Pseudarthrobacter siccitolerans* 4127T) showed a higher 16S rRNA sequence identity that is more than 98.27%. When comparing type strains, we found that *Arthrobacter* sp. PAMC25284 had ANI values higher than 95%, which is the algorithmic cut-off for species-level identification. *A. oryzae* KV-651T showed the highest ANI value of 99.75% (ANI coverage of 97.16%) with 16S rRNA gene (obtained from EzBioCloud) identity of 99.79%. *A. humicola* KV-653T showed the second highest 16S rRNA sequence identity (99.45%), which was obtained from the EzBioCloud with an ANI value of 99.2%. Table S1 of the Supplementary Materials (SM) lists details of the database search to identify the strain. As a result, the phylogenetic tree analysis and ANI values revealed the same clade, with the *Arthrobacter* sp. PAMC25284 having the closest relationship to *A. oryzae* KV-651T and *A. humicola* KV-653T.

| Features                | Value     |
|-------------------------|-----------|
| A: Genomic statistics   |           |
| Contings                | 1         |
| Total length bp         | 3,883,680 |
| N50                     | 3,883,676 |
| L50                     | 1         |
| GC%                     | 65.6      |
| B: genomic features     |           |
| Assembly level          | Complete genome |
| Chromosome genes        | 3,588     |
| Protein-coding genes    | 3,150     |
| Pseudogenes             | 366       |
| rRNA genes              | 19        |
| tRNA genes              | 50        |

Based on types of copper in proteins classified as type I copper, type II copper, and type III copper using secondary data [42]. Type I copper comprises a blue copper center, red Cu center, binuclear copper center, and type I copper center.
The twenty-nine sequences that are characterized as blue copper centers, type I copper, and variants from different origins along with different biological roles were used for the query. The phylogenetic tree was constructed
using neighbor joining alignment revealed that the strain PMAC25284 had a multi-copper oxidase domain-containing protein (as coded mco1, mco2, mco3, and mco4) clusters with different variants of type I copper centers, which is shown in Figure 3. It was found that mco1 and mco3 of strain PAMC25284 were out-border clustered with multicopper oxidase, CumA of *P. syringae pv. tomato* DC3000 (23% bootstrap support), whereas mco2 shared the same clade with bilirubin oxidase of *Albitiembria verrucaria* (65% bootstrap support). Moreover, it was revealed that mco4 of strain PAMC25284 shared the same clade with dihydroquinoline oxidase of *Aspergillus terreus* (21% bootstrap support). Even though multi-copper oxidase domain-containing genes were found in both Gram-positive and Gram-

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**Figure 2:** Neighbor joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain PAMC25284 (indicated with a red circle) and the type strains of related *Arthrobacter* species. The numbers at the nodes indicate the level of bootstrap support based on a maximum likelihood of 1,000 resampled datasets. Scale bar = 0.005 substitutions per nucleotide position. Accession numbers of the sequences are indicated in parentheses.
negative bacteria, another strain of Arthrobacter sp. PAMC25564 isolated from cryoconite [43] did not have any multi-copper oxidase domain-containing genes. Therefore, this study suggested that even if microorganisms are isolated from the same polar region, their functional genes differ depending on their habitat.

3.3. Multicopper Oxidase Sequence Analysis. The protein sequences of only six different strains were extracted as a model containing the residues considered significant in copper coordination, along with multi-copper oxidase domains incorporating protein sequences from the strain PAMC25284 (Figure 4), where the typical hallmarks of all MCOs were identified. The ligand groups coordinating the type I copper center in MCO are 1Cys and 2His residues [42]. MCO protein is identified by the presence of highly conserved histidine and cysteine rich signature sequences (HXXH, HXH, HXXHXXH, and HCHXXHXXXXX/L/F) inside the cupredoxin domain [44]. The bioinformatics tools like Signal P 5.0 predicted all multi-copper oxidase domain-containing proteins of strain PAMC25284 are intracellular, whereas the TMHMM Server 2.0 predicted a transmembrane domain in the C-terminal region of all multi-copper oxidase domain-containing proteins [38]. The genome of Arthrobacter sp. PAMC25284 possessed four multi-copper oxidase domain-containing gene clusters in downstream (Figure 5 and Table S2 of the Supplementary Materials), which have not been reported in Arthrobacter until now. Therefore, this study might be the first to report the occurrence of four multi-copper oxidase domain-containing genes in the genus Arthrobacter.
homeostasis are transcriptional regulators, chaperones and storage proteins, cell surface/secretory transporters and receptors, oxidoreductases, electron transfer/energy production/blue Cu proteins, free radical scavenging protein, oxidase, and monoxygenases [46].

Three core elements of copper homeostasis are present in both bacteria, Gram-positive and Gram-negative: a copper exporting ATPase (CopA), a copper chaperone (CopZ), and a copper-responsive transcriptional regulator (CopY) [47]. We have found similar core elements of copper homeostasis (CopA, CopZ, and CopY) in the strain PAMC25284, except CopY (Table 2). It is reported that additional defense against copper is provided by the periplasmic CueO-type multi-copper oxidases, which can oxidize Cu⁺ to less toxic Cu²⁺.
and catechol to copper-binding pigments in Gram-negative bacteria, which is intriguing information about Gram-positive bacteria strain PAMC25284 [48–50]. Twin-arginine translocase (Tat) export systems exist in both Gram-positive and Gram-negative bacteria [51], which export proteins across the cytoplasmic membrane in a posttranslational manner. Table S2 of the Supplementary Materials and Figure 6 summarize Tat export systems and other elements, along with the sequence similarity.

3.5. Copper Defense Mechanisms in Arthrobacter sp. PAMC25284. Copper transport into the cytoplasm was proposed to be mediated by the transmembrane protein, CopD, which has been characterized in P. fluorescens SBW25 [52]. Likewise, strain PAMC25284 also possesses CopD sequence but no similarity with the Gram-negative strains like P. fluorescens SBW25 and less similarity with E. coli DH5α (26.37%) (Table S3 of Supplementary Materials). Furthermore, Gram-positive bacteria, such as E. hirae ATCC9790 and M. tuberculosis H37RV, do not have CopD protein. Therefore, it is possible that our Gram-positive strain PAMC25284 has CopD protein along with CopZ protein. However, it had only 32.26% of its amino acid identity (homology) with Gram-positive strain E. hirae ATCC9790 and 35.94% with Gram-negative strain P. aeruginosa PAO1. Copper entering the cytoplasm is complexed by the CopZ-like copper chaperone, which directs it to regulators of gene expression and the CopA ATPases for export into the periplasmic space [47]. CopA was a member of the P-type ATPases superfamily. The strain PAMC25284 includes five different P-type ATPases (KY499_RS03705, KY499_RS04025, KY499_RS04195, KY499_RS04195, and KY499_RS12155) in different loci (obtained from the NCBI database); it was possible that it had CopA protein because the KY499_RS04025 locus sequence shares 41.66% (the highest) with CopA of E. hirae. Even though E. hirae ATCC9790 has two P-type ATPases like P. aeruginosa PAO1, only CopB had been demonstrated to confer copper tolerance [53]. CopB from E. hirae ATCC9790 was the first P-type ATPase whose transport in membrane vesicles was directly demonstrated using 64Cu+ [54]. CopB of E. hirae ATCC9790 shared 43.90% identity with the locus KY499_RS03705 of strain PAMC25284. Similarly, a MerR-type copper-responsive transcriptional activator, CueR, regulated the expression of two genes important for copper homeostasis (CopA copper efflux ATPase and the periplasmic CueO multi-copper oxidase). PAMC25284 also had five MerR-type copper-responsive transcriptional activators (KY499_RS05070, KY499_RS11345, KY499_RS13545, and KY499_RS13545). Among five MerR types, locus tag KY499_RS13545 found high similarity with CueR of E. coli DH5α, whereas with CueR of P. aeruginosa PAO1 had the highest similarity with locus tag KY499_RS11345. It has been shown that the connection between CopZ and MerR enhances CopA activation and copper sequestration in the periplasm [55].

The strain PAMC25284 has five multi-copper oxidase domain-containing proteins with varying loci (KY499_RS02210, KY499_RS04160, KY499_RS01525, KY499_RS03595, and KY499_RS04055). CueO of E. coli is a multi-copper oxidase that had robust cuprous oxidase activity that could contribute to copper resistance [49, 56]. One possible contribution of CueO to copper tolerance was the oxidation of toxic Cu+ to Cu2+ [48, 50]. A second
mechanism by which CueO could contribute to copper resistance was by the oxidation of siderophores and other phenolic compounds to their polyphenols [49]. Among them, the highest protein sequence similarity to CueO of *E. coli* DH5a was at the locus tag KY499_RS04055 (34.85%). A similar role in copper tolerance was also demonstrated for the MmcO of *M. tuberculosis* H37Rv [57]. MmcO has lipidation at Cys35 and is secreted by the Tat secretion system [50], which indicates that the protein may be membrane associated in the periplasm of *M. tuberculosis*. When compared to the strain PAMC25284 multi-copper oxidase domain-containing protein, the protein with locus tag KY499_RS04055 had the highest similarity of 40.61%. TatA, TatB, and TatC are the three proteins found in the *Arthrobacteria* Tat export system, which was like that of *M. tuberculosis* H37Rv [58–60]. TatA protein of PAMC25284 resembled TatA of *E. coli* DH5a by 29.87%, whereas TatA proteins of *P. aeruginosa* PAO1 and *M. tuberculosis* H37Rv by 28.57% and 50%, respectively. Similarly, TatB protein of strain PAMC25284 is similar to TatB of *E. coli* DH5a by 33.93%, whereas the TatB proteins of *P. aeruginosa* PAO1 and *M. tuberculosis* H37Rv were similar by 31.58%, and 27.05% respectively. The last subunit TatC protein of PAMC25284 resembled TatC of *E. coli* DH5a by 33.07%, whereas the TatC proteins of *P. aeruginosa* PAO1 and *M. tuberculosis* H37Rv by 34.39% and 35.64%, respectively. TatA and TatB proteins form a complex that contains the binding site for Tat preproteins [61, 62]. After a preprotein binds to TatBC, TatA protein was recruited to the complex [61]. TatA protein was generally believed to form an export channel and is found in homo-oligomers of varying sizes, which may give the Tat export the flexibility to export folded proteins of different sizes and shapes [63–65].

4. Conclusions

In summary, we elucidated the complete genome sequence of polar *Arthrobacter* sp. PAMC25284 and compared it to copper resistance genes characterized by nonpolar Gram-positive and Gram-negative strains. The polar *Arthrobacter* sp. PAMC25284 was isolated from seawater under laboratory conditions and confirmed it by analysis of 16s rRNA sequences. Even though this strain has been previously isolated from harsh and noncontaminated conditions, there are no reports of copper genes being employed in such a cold environment. The genome of *Arthrobacter* sp. PAMC25284 is 3.89 Mb in size and has a GC content of 65.6%, indicating that this strain has high GC content despite its small genomic size. The copper-transporting ATPase, a copper chaperon, and copper-responsive transcriptional regulators associated with copper resistance genes are all described for the first time in *Arthrobacter* species. We confirmed that PAMC25284 has 5 P-type ATPases, 1 TatABC translocation system (TatABC), 1 copper chaperone, 5 transcription factors (Merr), 5 multi-copper oxidase proteins (MCO), and 2 copper uptake systems. Further functional analysis of the identified genes might give insights into the detailed molecular mechanisms of cold-adapted microbes to tolerate and transform copper in copper-contaminated environments. This study provides a foundation to understand how the Gram-positive strain PAMC25284 produces metal-binding molecules to maintain proper metal homeostasis that has allowed bacteria to colonize various extreme environments, like Antarctica.

Data Availability

The 16S RNA datasets analyzed during the current study are available in the EzBioCloud repository and NCBI database, accession numbers: MN559964 for *Pseudarthrobacter psychrotolerans* YJ56, KF212463 for *Arthrobacter ginsengisoli* DCY81, AF235091 for *Pseudarthrobacter sulfinivorans* ALL, X80741 for *Pseudarthrobacter polychromogenes* DSM20136, X83408 for *Pseudarthrobacter oxydans* DSM20119, AF330692 for *Pseudarthrobacter scleromycetous* YH-2001, KF150696 for *Arthrobacter bambusae* GM18, X80743 for *Paenarthrobacter nitroreducens* DSM420, X83406 for *Paenarthrobacter histidinolovorans* DSM20115, BJMD01000050 for *Paenarthrobacter aurescens* NBC12136, AJ512504 for *Paenarthrobacter nitroguaiacolicus* G2-1, CAQI01000001 for *Pseudarthrobacter siccitolerans* 4J27, CP002379 for *Pseudarthrobacter phenanthrenivorans* Sphe3, CP001341 for *Pseudarthrobacter chlorophenolicus* A6, LT629779 for *Pseudarthroncter equi* IMMI1-1606, AM409361 for *Paenarthrobacter defluviil 4Cl-α*, AB248526 for *Paenarthrobacter niagatensis* LC4, MK211245 for *Pseudarthrobacter sp.* T11b, JF421614 for *Pseudarthrobacter enclensis* NIO-1008, AOFD01000111 for *Arthrobacter nitrophilicus* SJCon, KM507333 for *Arthrobacter pokkali* P3B162, KJ082091 for *Arthrobacter liui* DSXY973, X80740 for *Arthrobacter pascens* DSM20545, BAEG01000072 for *Arthrobacter globiformis* NBC12137, AB279890 for *Arthrobacter humicola* KV-653, AB279889 for *Arthrobacter oryzae* KV-651, NZ_CP080382 for *Arthrobacter sp* PAMC25284, GC_F90009975.1 for *Arthrobacter cupressi* CGMCC1.10783, MH063435 for *Arthrobacter celeriacrescens* NEAU-SA2, BCQN01000021 for *Arthrobacter woluwensis* NBC107840, KF479547 for *Arthrobacter nanjingensis* A33, KP128918 for *Arthrobacter ginkgopsis* SYP-A7299, LC065376 for *Zafaria choliastenensis* NCCP-1664, AB778264 for *Zhihengiuella salsuginis* NBC109062, BJNY01000040 for *Glutaminibacter uratayodans* NBC15515, NZ_FPCG00000000 for *Micrococcus terrus* CGMCC1.7054, GJ791777 for *Citococcus nitrophilicus* PNP1, AJ344143 for *Citococcus muralis* 4–0. NC_002516.2 for *Saccharomyces cerevisiae*, NP001354161.2 for *Hephaestin* 

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**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

T-JO designed and supervised the project. JK, PS, S-RH, HP, and T-JO wrote the manuscript. All authors discussed the results, commented on the manuscript, and approved the manuscript.

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**Supplementary Materials**

Supplementary Materials: The matching results of EzBioCloud database, multi-copper oxidase domain protein gene clusters in the SEED database and protein sequences similarity for the strain PAMC25284 are shown in Tables S1, S2, and S3, respectively. ([Supplementary Materials](#))

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