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Comparative Genome and Protein Analysis for Acidithiobacillus Ferrooxidans Strains in Terms of Heavy Metal Binding as an Application for Biomining

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Abstract

The majority of microorganisms cannot endure extreme conditions, including heavy metals, high salinity, acidity, alkalinity, and various stress factors. However, only a few can endure extreme environments, with the genus Acidithiobacillus being widely recognized. Studies have been conducted to demonstrate how proteomic alterations occur in reaction to heavy metals like copper, zinc, nickel, cadmium, potassium, iron, molybdenum, and arsenic. Entire genome sequencing of various Acidithiobacillus species has revealed new understandings of their roles. Four distinct functional gene categories were thoroughly examined for their capacity to bind heavy metals in the context of biomining applications. The complete genome sequence acquired was annotated and examined with Ezbiocloud software. Subsequently, utilizing the 16S rRNA gene sequence of the A. ferrooxidans YNTRS-40 strain, the sequence's purity was evaluated through cont16s rRNA provided by EZbiocloud. Based on the phylogeny derived, two extremes were noted: one being the nearest strain, A. ferrooxidans ATCC 23720, and the other being the outgroup, A. albertensis DSM 14366. Twenty-two distinct heavy metal binding genes were examined using RAST annotation software. The genes included were Mod, ZnuA, ZnuC, MerP, SufA, PstB, PstS, PhnK, PhnL, FeoB, MntH, MerC, MgtC, and PhoU. The analyzed bacteria with these genes were recognized as having the potential for application in heavy metal bioremediation.

Keywords: A Ferrooxidans, Biomining, Heavy Metal, Binding, Genes.

Introduction

Biomining is the method of extracting metals from ores and other solid substances, usually employing prokaryotes, fungi, or plants [1]. These organisms produce various organic compounds that bind metals from the surroundings and transport them back to the cell, where they are generally utilized to coordinate electrons. Among these microorganisms, Acidithiobacillus ferrooxidans stands out as a potential candidate [2]. The majority of microorganisms do not endure extreme conditions such as heavy metals, salinity, acidity, alkalinity, and stress. Nevertheless, certain microorganisms can endure in these harsh conditions, one of which is the Acidithiobacillus genus of bacteria. It has been stated that Acidithiobacillus plays a crucial role in the cycling of nutrients related to metals in the environment and can help in cleaning up metal-polluted sites by oxidizing and reducing these metals [3, 4]. The Acidithiobacillus genus includes approximately nine recognized species such as A. ferrooxidans, A. albertensis, A. ferridurans, A. ferriphilus, A. ferrivorans, A. sulfuriphilus, and A. thiooxidans. Among these genera, A. ferrooxidans is the dominant one, featuring various species such as A. ferrooxidans NTRS-40 and A. ferrooxidans ATCC 23720.

From A. ferridurans, A.ferridurans ATCC33020 is the recognized variant. A. ferrivorans NO-37 is a common variant of A. ferrivorans. A.ferrianus MG and A.ferriphilus M20 are likewise the species present in the Acidithibacillus genus of A.ferriansus and A.ferriphilus. Among these, the most researched microorganisms is A. ferrooxidans due to its capabilities to oxidize iron and sulfur, possible environmental adaptability, and extensive applications [5].

Acidithiobacillus ferrooxidans is a gram-negative, aerobic chemolithoautotrophic bacterium that thrives in acidic conditions. It acquires the energy needed for growth through the aerobic oxidation of Fe+2 (ferrous iron) and H2S (reduced sulfur) compounds into Fe+3 (ferric iron) and H2SO4 (sulfuric acid), correspondingly. It can additionally utilize hydrogen or formate when Oxygen is present. Moreover, under anaerobic conditions, it can reduce ferric iron using sulfur or hydrogen as electron donors [6]. It possesses distinctive metabolic functions. This resulted in it being one of the key bacteria for leaching heavy metals such as gold, copper, uranium, and cobalt from sulfide ores [7]. The unusual energetic metabolism of A. ferrooxidans is crucial to comprehend its bioleaching potential [8].

It is challenging to research A. ferrooxidans genetically. As a result, several proteomics and genomes investigations of A. ferrooxidans have been conducted in an effort to learn more about the processes underlying adaptability to environmental changes. Research has been done to find out how heavy metals like copper, zinc, nickel, cadmium, potassium, iron, molybdenum, arsenic, and others affect the proteome. A comparative proteomic and genomic approach was used to compare different species of A. ferrooxidans in order to gain insight into how the bacterium can withstand damage from heavy metals and how it binds to metals to oxidize or reduce [9, 10].

In A. ferrooxidans, researchers have described a number of respiratory chain models, most notably iron-based energy metabolism. These theories were widely accepted and included the rus operon that encodes two c-type cytochromes, Cyc1 and Cyc2, an aa3-type cytochrome oxidase, rusticyanin, and an open reading frame (ORF) of unknown function. Results on the Sulphur respiratory chains have only recently been reported due to the complexity of the energetic metabolism of reduced Sulphur compounds [11, 12].

New information on the roles of several Acidithiobacilli species has been made possible by whole genome sequencing. A. ferrooxidans YQH-1's complete genome sequencing has shown a large number of genes linked to carbon dioxide and dinitrogen fixation, pH tolerance, oxidative stress, heavy metal binding, and heavy metal detoxification [13]. Another work used whole-genome sequencing analysis and a bioinformatics technique to show sulphur oxidation in the extremophile Acidithiobacillus thiooxidans. Different researchers have employed transcriptional analysis to investigate the substrate regulation and gene identification of psychrotolerant Acidithiobacillus ferrivorans throughout a bioleaching process, allowing functional predictions made by genome analysis to be further evaluated by experimental methods. Little is known about the minerals created during the bioleaching process, despite the fact that metabolic routes for iron oxidation have been thoroughly investigated [14].

The comparative genome and proteome analysis of the A. ferrooxidan strain in terms of heavy metal binding has been addressed in this study. The whole genome sequence and annotation of A. ferrooxidan were used for this activity, and bioinformatics analyses of the genome of A. ferrooxidan allowed for the identification of numerous candidate genes related to heavy metal binding as a biomining application. Several of these putative genes were characterized in the present study by measuring their transcriptional expression profiles and functionality.

The remaining parts of the paper is organized as follows. In section 2 materials and methods is presented. Section 3 contains results and discussion of the study. Finally conclusion is drawn in Section 4.

Materials and Methods

Genome Overview

The whole genome sequence obtained was annotated and analyzed using Ezbiocloud software. Next, using the A. ferrooxidan YNTRS-40 strain's 16S rRNA gene sequence, the purity of the sequence was analyzed by cont16s rRNA from EZbiocloud. Then two fragmented sequence obtained. One of the fragmented sequences was run blastn using NCBI Blastn software online. Then the top seven hits (100% similarity) were obtained and used for phylogenetic tree construction [15-17].

Phylogenetic Tree Construction

Using the top seven best hits of blastn results (100% similarity), phylogenetic tree was constructed using MEGA 11 software. MEGA11 software (CLUSTALW) was used to perform multiple alignment phylogenetic tree construction using likely hood method. Sequence alignment and average nucleotide identity (ANI) was analyzed using OnthoANI (OAT) software.

Functional Gene Analysis

The functional genes analysis was done using Rapid annotation using subsystem technology (RAST). To assign functions to the genes, predict subsystem in the genome and to predict metabolic functions of the assigned genes, Rapid Annotations using Subsystems Technology (RAST) was used. So, in this study RAST was used to analyze functional genes for heavy metal binding as an application for biomining. The RAST uses FIG FAM protein family subset to predict gene functions. Then functional genes used for heavy metal binding as an application for biomining. Finally, the new strain formed would be described and housekeeping gene was identified [18].

Results and Discussion

Results

Genome Overview

With known genome assembly and base pairs arranged into two contigs (Table 1), the genome of the investigated Acidithiobacillus ferrooxidans YNTRS-40 strain was examined to look into its traits and activities [19]. When the genome was compared to other known Acidithiobacillus ferrooxidans genomes, the OrthoANI value for Acidithiobacillus ferrooxidans ATCC 23720 was 100%, while the OrthoANI value for A. albertensis DSM 14366 was at least 97.72% (Figure 2). The substantial genetic variability in the A. ferrooxidans YNTRS-40 genome, which may be the consequence of long-term horizontal gene transfer or adaptation to severe environmental circumstances like pH stress, is probably the cause of the comparatively low pairwise nucleotide identity seen.

Table 1: Genomic Information of A ferrooxidans Strain YNTRS-40

S/N	Bacteria	Genome size	G+C	CDS	Contigs	rRNA	tRNA	Gene size	Ref
1	A.ferro-oxidan YNTRS 40	3,257,037	58.5%	3,283	2	6	46	3,339	[6]

Analysis of 16S RRNA Gene

For the bacterium under investigation (A. ferrooxidans NTRS-40), accession number NZ_CP040511.1, the 16S rRNA gene sequence was acquired. With similarity values of 100%, a comparison of this sequence with closely related strains that were deposited in public databases verified that the strain A. ferrooxidans ATCC 23720 was associated with the species being studied.

A. albertensis DSM 14366 had the lowest similarity of any strain in the database, at 97.72%, which was below the threshold level to be classified as belonging to the species. All other strains in the database had similarities that were lower than this. So, based on the 16S rRNA gene, the phylogenetic position of A.albertensis DSM 14366 shown in Figure 1, is separated in an independent branch (outgroup) [20].

Phylogenetic Construction

Whole genome sequence was annotated using RAST (Rapid Annotation subsystem technology). For the construction of phylogenetic tree, first 16S rRNA gene sequence of A. ferrooxidan YNTRS-40 strain was fragmented by using database cont16S rRNA from Ezbiocloud. Then the obtained result was run blastn and finally phylogenetic tree was constructed by MEGA11 software using CLUSTAL W to perform a multiple alignment. The neighbor-joining method was used to construct a phylogenetic tree. During the construction of phylogenetic tree, Bootstrap values were calculated by MEGA 11 using likely hood method. Sequence alignment (SA) and Average Nucleotide Identity (ANI) analysis was performed using OAT software. Genome annotations of bacteria under study revealed the presence of various genes for iron (Fe) and sulfur (S) metabolism. In addition to this, aromatic compound degradation, stress response and metal resistance genes obtained (Table 4) [21].

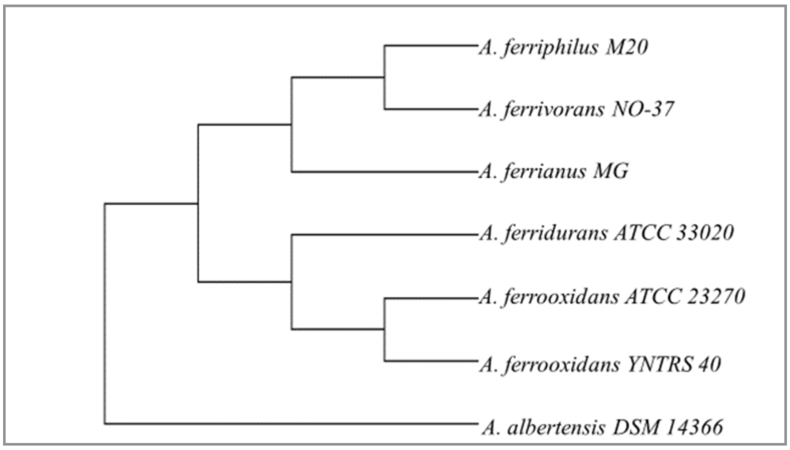

Figure 1: Phylogenetic tree of a. Ferrooxidansstrain yntrs-40 with reference strains obtained from database

Genomic Nucleotide Analysis (Gene Ontology) (ANI)

The gene ontology and genomic nucleotide analysis (ANI) showed the presence of different features compared with other available strains obtained from NCBI.

Table 2: General features of A.ferrooxidans NTRS-40 genome used in this study including six strains available on NCBI

strain	Genome size (bp)	G+C	Contigs	CDSs	rRNA	tRNA	Hit%
A.fxdn NTRS-40	3,257,037	58.5%	2	3,283	6	46	100
A. fxdn ATCC 23270	2,982,397	58.8	1	3,042	6	78	100
A.frvorns No-37	3,207,552	56.6	1	3,250	6	47	100
A.fdur ATCC33020	2,921,399	58.4	1	3,033	6	46	100
A.ferriphilus M20	2,667,881	58.4	127	2,641	6	46	100
A. ferrianus MG	316,586	58.2	90	3,083	7	47	100
A. al DSM 14366	3,503,318	52.5	141	3,596	6	48	100

After the analysis of 16S rRNA gene sequence using EZBiocloud software, different categories of clustered strains of the bacterium has been seen. These were A. ferriphilus M20, A. ferrivoransNO-37, A. ferridurans ATCC33020, A. ferrooxidans ATCC 23270, A. ferriansus MG and A. albertensis DSM 14366.

This result was similar to a study done by [22]. ANI analysis showed that there is strong similarity between the strain under study and Acidithiobacillus ferrooxidans ATCC 23720 (Figure 2 and Table 3).

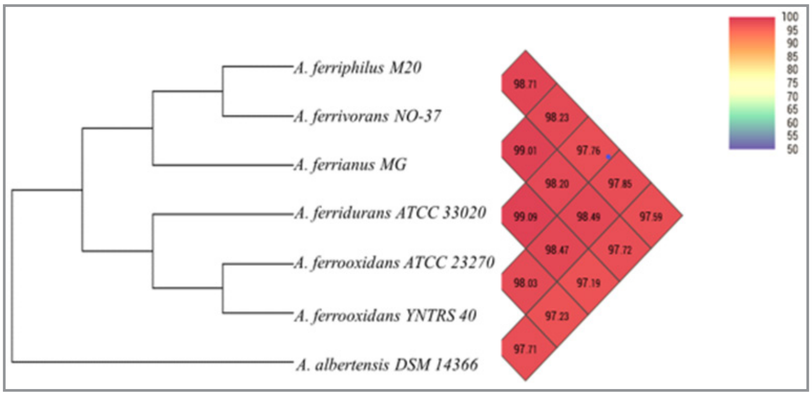

Figure 2: Gene Onthology of A. ferrooxidans

Figure 2 shows a heatmap and dendrogram illustrating the genetic clustering of Acidithiobacillus strains, highlighting their similarity levels and diversity, aiding in microbiology and evolutionary biology research.

Table 3: Average Nucleotide Identity Analysis of Acidithiobacillus

STRAIN	MG	DSM 14366	M20	NO-37	ATCC 23270	YNTRS 40	ATCC 33020
A. ferrianus MG							
A. albertensis dsm 14366	97.71						
A. ferriphilus m20	98.47	97.19					
A. ferrivorans no-37	98.03	97.23	99.09				
A. ferrooxidans atcc 23270	97.85	97.59	98.23	97.76			
A. ferrooxidans yntrs 40	97.93	97.72	98.18	97.84	100		
A. ferridurans atcc 33020	98.49	97.72	99.01	98.2	98.71	98.7	

Genomic Analysis of Heavy Metal Binding, Transport and Resistance Genes

The genome of Acidithiobacillus ferrooxidans NTRS-40 revealed different genes related to heavy metal binding and responsible for utilization as energy source. Sometimes these genes were lead in the activity of biomining as a result of bioremediation of heavy metals and toxic elements. During the analysis different heavy metal binding genes were identified using RAST software [23]. Among the genes analysed using the software, some of them are listed (Table 4). Up on the analysis 22 genes of heavy metal or element binding genes or proteins were identified as in bacteria under study. Out of these, 5 of them have no specific genes, only binding proteins were identified. They were 3 4Fe-4S ferredoxin, iron-sulfur binding proteins and 2 heavy metal binding proteins. From the identified genes, 3 genes were Molybdenum binding (ModC and 2Mod A), 2 Zinc binding (ZnuA and ZnuC), 7 Iron binding (TonB, 2HesB_2IscA_2SufA), 1 Mercury binding (MerP), and 4 Phosphate binding genes (PstB, PstS, PhnK and PhnL) (Table 4 and Figure 3).

Large number of genes responsible for resistance to toxic compounds and heavy metals were identified using genome analysis of the bacterium (Table 4). A number of genes important in heavy metal resistance and binding were observed in the membrane transport (ABC transporter system) and periplasmic membrane (Figure 3).

Heavy metal transport system proteins and genes analysed from the bacterium under study was Iron and ferric transport genes and proteins (TonB, FeoB and TonB-dependent receptor), magnesium and cobalt transport protein (CorA), Managanese transport protein (MntH), Mercuric transport protein (MerC), Mg(2+)-transport-ATPase-associated protein (MgtC), molybdenum ABC transporter ATP-binding protein (ModC), molybdenum ABC transporter, substrate-binding protein (ModA), molybdenum transport system protein (ModD), phosphate ABC transporter, ATP-binding protein (PstB), phosphate ABC transporter, permease protein (PstA), phosphate ABC transporter, permease protein (PstC), phosphate ABC transporter, substrate-binding protein (PstS), phosphate transport system regulatory protein (PhoU), phospholipid ABC transporter shuttle protein (MlaC), potassium-transporting ATPase A chain (EC 3.6.3.12) (TC 3.A.3.7.1), potassium-transporting ATPase B chain (EC 3.6.3.12) (TC 3.A.3.7.1), potassium-transporting AT-

Pase C chain (EC 3.6.3.12) (TC 3.A.3.7.1), sodium-transporting ATPase subunit G, zinc ABC transporter, ATP-binding protein (ZnuC), zinc ABC transporter, permease protein (ZnuB), zinc ABC transporter, substrate-binding protein (ZnuA) and Copper-translocating P-type ATPase EC 3.6.3.4 for transporting lead, cadmium, zinc and mercury. In addition to heavy metal transport system, there were heavy metal resistance genes and proteins analyzed from genome of the A.ferrooxidans NTRS-40 bacterium like Arsenic resistance protein (ArsH), Cobalt/zinc/cadmium resistance protein (CzcD), Cobalt-zinc-cadmium resistance protein, Copper resistance protein (CopC), Copper resistance protein (CopD) and Mercuric resistance operon regulatory protein (MerR).

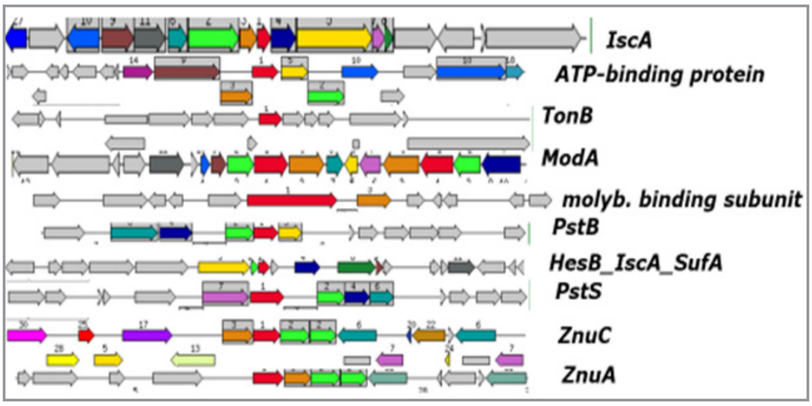

Figure 3: Functional Gene Annotation for Acidithiobacillus Ferrooxidans with Genes Involved in Heavy Metal Binding

Table 4: Lists of Genes and Proteins Involved in Heavy Metals and Elements Binding Analysis of Acidithiobacillus Ferrooxidans NTRS-40

Heavy metal and element binding genes and proteins	
Gene	**Proteins**
ModC	Molybdenum-binding protein
ModA	Molybdenum ABC, periplasmic molybdenum-binding protein
ZnuA	Zinc ABC, periplasmic-binding protein
ZnuC	Zinc ABC, ATP-binding protein
IscA	Iron binding protein for iron-sulfur cluster assembly
TonB	Ferric siderophore, periplasmic binding protein
HesB_IscA_SufA	probable iron binding protein
HesB_IscA_SufA	probable iron binding protein in Nif operon
MerP	Periplasmic mercury(+2) binding protein
PstB	Phosphate-binding protein
PstS	Phosphate ABC, periplasmic phosphate-binding protein
PhnK, PhnL	Phosphonates-binding protein

Table 4 shows different genes and the proteins they produce, which are involved in binding and transporting various essential elements and heavy metals across the cell membrane. These proteins play important roles in cellular processes and help the cell adapt to different environmental conditions. Each gene is associated with a specific protein and its function, such as binding molybdenum, zinc, iron, and mercury, phosphate, and phosphonate compounds for transport or utilization within the cell.

The chromosomal region of the focus gene (top) is compared with similar organisms. The graph is centered on the focus gene, which is red and numbered and denoted 1. Sets of genes with similar sequence are grouped with the same number and color. Genes whose relative position is conserved in at least four other species are functionally coupled and share gray background boxes. As it was shown by Rasta viewer software, focus gene always points to the right (Figure 3 and 4).

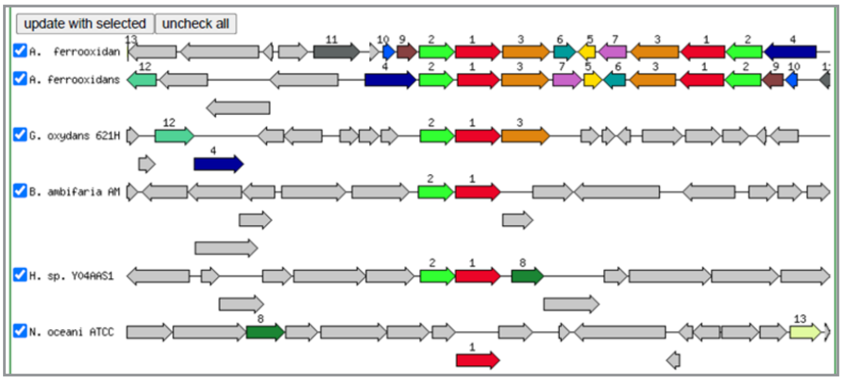

Figure 4: Molybdenum ABC transporter, substrate-binding protein ModA

Table 5: Heavy metal transporter and binding proteins and their associated gene

heavy metal transporter and binding proteins and their associated gene	
Genes	**Proteins**
TonB	Ferric siderophore transport system, periplasmic binding protein
FeoB	Ferrous iron transporter
CorA	Magnesium and cobalt transport protein
MntH	Manganese transport protein
MerC	Mercuric transport protein,
MgtC	Mg(2+)-transport-ATPase-associated protein
ModC	Molybdenum ABC transporter ATP-binding protein
ModA	Molybdenum ABC transporter, substrate-binding protein
ModD	Molybdenum transport system protein
PstB	Phosphate ABC transporter, ATP-binding protein
PstA, PstC	Phosphate ABC transporter, permease protein
PstS	Phosphate ABC transporter, substrate-binding protein
PhoU	Phosphate transport system regulatory protein
MlaC	Phospholipid ABC transporter shuttle protein
ZnuC	Zinc ABC transporter, ATP-binding protein
ZnuB	Zinc ABC transporter, permease protein
ZnuA	Zinc ABC transporter, substrate-binding protein
Not Identified	ABC-type Fe3+-siderophore transport system permease component
Not Identified	Heavy metal transport/detoxification protein
Not Identified	Lead, cadmium, zinc and mercury transporting ATPase (EC 3.6.3.3) (EC 3.6.3.5); Copper-translocating P-type ATPase (EC 3.6.3.4)
Not Identified	Potassium-transporting ATPase A chain (EC 3.6.3.12) (TC 3.A.3.7.1)
Not Identified	Sodium-transporting ATPase subunit G

Table 5 indicates various genes and the proteins they produce, which are involved in transporting essential elements and heavy metals across bacterial membranes which were summarized from RAST viewer. These proteins play crucial roles in the uptake, transport, and detoxification of substances like iron, magnesium, cobalt, manganese, mercury, molybdenum, phosphate, phospholipids, and zinc. They also contribute to the adaptation and survival of bacteria in different environments. Each gene is associated with a specific protein and its function, such as transporting specific ions or molecules across the bacterial membrane.

Table 6: Heavy metal Resistance genes and associated proteins

Heavy metal Resistance genes and associated proteins	
Genes	**associated proteins**
ArsH,	Arsenic resistance protein
CzcD,	Cobalt/zinc/cadmium resistance protein
CzcD	Cobalt/zinc/cadmium resistance protein

CopC	Copper resistance protein
CopD	Copper resistance protein
MerR,	Mercuric resistance operon regulatory protein

Table 6 describes genes and the proteins they produce, which are involved in heavy metal resistance. These proteins play crucial roles in protecting organisms from the toxic effects of these heavy metals, allowing them to survive and adapt in environments with high metal levels. Each gene is associated with a specific protein that helps the organism resist a particular heavy metal, and their functions are essential for the adaptation and survival of the organisms in challenging conditions.

Discussion

The A.ferrooxidans NTRS-40's genome was analysed using different software like EZBiocloud, CLASTAL W, OAT, MEGA 11 and others. The result has confirmed the presence of arrangement of genes and proteins involved in heavy metal resistance. Some of them were metal-binding proteins, metal transporters, metal chelators, metal reductases, and metal-resistant genes. In addition, there were also genes and proteins capable of binding, transporting, reducing and oxidizing for the survival and resistance of the bacteria, which is in the same result with.

For the average nucleotide identity or ortho OAT ANI analysis and phylogenetic characterization of the Bacterium of the study, the results indicated a relationship with the A.ferrooxidans ATCC 23720. 16S rRNA gene phylogeny was studied to evaluate the taxonomic position of the bacterium under study within other members, showing a close relationship with A. ferridurans (98.18%), A. pherriphilus (98.18%), A. ferrianus (97.93%), A. ferrivorans (97.54%) and A. albertensis (97.73). This phylogenetic tree showed that A.ferrooxidans ATCC 23720 has 100% similarity with bacterium under study. This showed both bacteria were categorized in the same species. But A. albertensis DSM 14366 showed the least similarity with A.ferrooxidans NTRS-40. But according to [10, 21, 23], bacterial strain A.albertensis DSM 142366 is not outgrouped from the branch.

According to the phylogenetic tree, this bacterium was found in outgroup branch of the phylogeny. The bacterium A.ferrooxidans NTRS-40 was branched together with A.ferrooxidans ATCC 23720 with the highest value (100%), indicating it was closely related to the A.ferrooxidans NTRS-40.They also categorized under the same species when other bacterial groups in this study were in different species.

In response to heavy metals resistance, the bacterium has developed different genetic mechanisms like heavy metals binding proteins and genes, heavy metals transporting proteins and genes and also heavy metals resistance genes and proteins. This was showed the bacterium can grow and remediate the toxified soils and other environments. A.ferridurans ATCC33020 was also reported to have tolerance to heavy metals and capable to remediate the soils as well as environments [24].

When bacterial groups associated with bacteria under study (A. ferrooxidans NTRS-40) in the phylogenetic tree and Ortho ANI were compared, only A.ferroxidans ATCC 23720 strain was related with functional genes during functional gene analysis by RAST software. When compared between the two strains with functional gene analysis, A.ferrooxidans NTRS-40 had more functional genes of heavy metal resistance and binding genes. So, from comparative analysis, these bacterial strains (A. ferrooxidans NTRS-40 and A.ferrooxidans ATCC 23720) are highly involved in biomining and heavy metal binding. This indicated that they play a great role in biomining of heavy metals as heavy metal bioremediation activity. This is supported by the two strains have similar functional genes ZunC and PstB which involves in binding of Zink and Phosphate compound [25]. In previous study, it was known that the bacterium under study has increased levels of proteins. These proteins were related to LPS synthesis which could be of great importance for heavy metal resistance. Due to this, various microorganism like Pseudomonas aeruginosa can accumulate metals on the cell surface through electrostatic interactions with LPS present in its membrane.

Table 4, 5 and 6 describes different genes and the proteins they produce, which are involved in binding and transporting essential elements and heavy metals across cell membranes, bacterial membranes, and providing resistance to heavy metals. These proteins play vital roles in cellular processes, bacterial adaptation, and protecting organisms from the toxic effects of heavy metals, enabling survival in challenging environments. Each gene is associated with a specific protein and function, such as binding, transporting, or providing resistance to particular ions or molecules, essential for cellular and bacterial survival and adaptation.

Conclusions

Genomic and functional analysis of A. ferrooxidans ATCC NTRS-40 revealed that they are highly capable of avoiding the toxicity of heavy metals in their surroundings. The majority of the heavy metal-exposed proteins in A. ferrooxidans NTRS-40 are linked to other bacterial strains, especially A. ferrooxidans ATCC 23720. Given the challenges in doing conventional genetic research on this microbe, bioinformatics analysis of the entire genome of A. ferrooxidans NTRS-40 offers a useful tool for gene identification and functional gene prediction. A comprehensive understanding of this bacterial gene composition, transport systems, heavy metal resistance, heavy metal binding, and metabolic capacity can be obtained by a genome study. These cellular functions underpin the essential traits of A. ferrooxidans that relate to its application in commercial biomining, such as its capacity to oxidize iron and sulphur, withstand low pH, and survive in settings that contain potentially hazardous organic and inorganic substances. Additionally, they imply that it can move metals from the cell to the surroundings, which would help the bioleaching of copper, zinc, mercury, molybdenum, cobalt, and other heavy metals. Additionally, the research generated a number of predictions that may prove helpful in understanding biomining systems.

Finally, the study reviewed that A.ferrooxidans ATCC 23720 is the most closely related strain to the bacterium under study, with 100% similarity in average nucleotide identity (ANI). Additionally, further researches will be important to analyze the practical application of these identified functional genes in heavy metal binding, transporting and resisting heavy metals in different areas, such as industries.

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of Data and Materials

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

The Submitted Version of the Paper was Approved by all Authors who Contributed Equally.

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