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DMRP: construction and bioinformatics analysis of databases for metal - related proteins in *Acidithiobacillus ferrooxidans*

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Abstract

A.ferrooxidan as one of the main biological leaching ore bacteria，has great application value in industrial leaching ore,has been the research hotspot in recent years. Studies on the existing

A.ferrooxidan is associated with a variety of metal ions: arsenic, aluminum, cobalt, strontium, chromium, copper, mercury, lead, lithium,etc. So far, *A.ferrooxidan* leading research has formed a lot of experimental data,including *A.ferrooxidan* the basic information of the nucleic acid,protein and enzyme, genome,transcriptome and proteomics data and dynamic data,etc. Therefore, the existing *A.ferrooxidan* metal-related protein data collection and mining, constructing special *A.ferrooxidan* metal-related protein database, set up a corresponding biological leaching mining research platform is the problem to be solved, in order to facilitate *A.ferrooxidan* biological leaching to provide better support for the further development of research.

Keywords: *Acidithiobacillus ferrooxidan*; metal - related proteins; databases

1. Introduction

A.ferrooxidan is a kind of typical autotrophic bacteria,belong to gram-negative

bacteria,aerobic acidophilus,widely exists in the metal mining area and biological

leaching mining system(Levicán et al., 2008). *A.ferrooxidan* is one of the most widely biological leaching used strains,it through the ferrous oxide,sulfur,and also types of sulfide to obtain energy(Raquel Quatrini et al., 2007).*A.ferrooxidan* in biological leaching plays a very important role, is often used for industry recycling other metals such as copper, cadmium, and the bacteria have tolerance to high concentrations of heavy metals, such as copper, zinc, arsenic and uranium(Zhang et al., 2013). Currently, metal toxicity is hindered bioleaching efficiency of the most serious limiting factor(Lombardi & Garcia, 2002). Therefore, the study of molecular mechanisms *A.ferrooxidans* tolerate high concentrations of heavy metals is a serious problem. If the resistance mechanism has been understood, we can reconstruct a wild strain to obtain a high concentration of heavy metal ions resistance. In addition, we can build engineered bacteria with a high concentration of heavy metals resistance by molecular techniques(He et al., 2006). *A.ferrooxidans* as the main bacterial leaching plays a vital role in biological metallurgy, physical activity and related bacteria chemistry is closely related to biological leaching efficiency(Olivieri et al., 2012). If the processing of the metal ion concentration in the sample exceeds the tolerance level *A.ferrooxidans* of bioleaching will be difficult to carry out. Therefore,further research of heavy metal tolerance and resistance

mechanisms *A.ferrooxidans* will provide theoretical guidance for further biological breeding(Barbosa et al., 2015).

In recent years,more and more attention was paid to metalloproteins.The proteins involved in the metal binding,transporting and metabolism have aroused much interest(Quatrini et al., 2005). Metalloproteins account for one third of the natural proteins, which perform important biological functions in living organisms. Nearly every protein needs to form either compounds with other proteins to implemennt its function, and metal ions are associated with an estimated 30–40% of all proteins, often in essential structural or functional roles(Song et al., 2014).

In the highly sophisticated scientific instruments essence of life it seems within reach, but very unfortunately in reductionist thinking under the guidance of the theory of biological rule, the secret of life is still a mystery: how to contain large amounts Heavy Metals in the environment to survive and metabolic and other issues are still a mystery. Study the scientific issues of great significance to the application environment of heavy metals bioleaching and bio-governance(Chen et al., 2011). However, so far around *A.ferrooxidans* existing knowledge is formed by a dizzying messy information system, at any point in the research work has not yet formed a comprehensive scientific research conclusion, a large number of scattered data has not been effectively integrated, not comprehensive and systematic understanding. Faced explore these unknown problems, we need to set a variety of advantages to cross-disciplinary integration of technology, research on the overall level of the phenomenon of life, the study of biological systems in order to view the relationship between the components of the system among all. Over all biological research, but also on the non-linear asymmetric complex biological giant open system dynamic, comprehensive understanding of quantitative, only the gradual accumulation of biological data, the full integration of existing data, information and knowledge can be more comprehensive understanding of the biological the behavior and mechanism(Cheng et al., 2014). Therefore, insight into the key role of regulatory mechanisms in a variety of different systems *A.ferrooxidans* played, on the need to study the whole biological systems. First, the need for existing research data, system integration, based on the data mining, the establishment of all kinds of related *A.ferrooxidans* bioinformatics databases, bioinformatics methods to analyze and forecast the entire system, from molecules to cells, from genes to the depth and breadth of the industrial application of a combination of in-depth exploration.

This study by building acidophilic thiobacillus ferrous metal oxide associated protein

database, integration of the existing research data, starting from the target system requirements, the content of the database design, the work can not only comprehensive insight into the research progress in this area, increase the depth study, but also a research platform for follow-up study to lay the information. In this study, we constructed a metal-related proteins database of *A.ferrooxidans*, integrated the existing data and designed the content of the database from the target system requirements. This work not only provides a comprehensive insight into the research progress of this field, , But also for future research to lay the information research platform.

2. Materials and methods

We established the first *A.ferrooxidans* metal-related protein database DMRP, and through the integration of network resources and secondary literature mining, increase the depth and breadth of data, improve data quality and practicality. Users can through the website:http://www.eamsyslzb.com/Default.aspx. We can intuitive and efficient brows the data retrieval, in order to provide greater support for *A.ferrooxidans* metabolism and bioleaching research.

3. Results and Discussion

3.1 Database construction

The main data in the database from NCBI (National Center for Biotechnology Information), the auxiliary supplement to literature data, and identification data authenticity. Important note information reference in protein sequence information and literature mining. Reference in the protein sequence information and literature data mining the annotation of important information. NCBI database is GenBank, DDBJ and EMBL data composed of three parts, integration of a very rich data resources, which is an ideal source of our data acquisition(Binkley et al., 2014).

We get original data by retrieval keywords,such copper, mercury, nickel, arsenic,zinc and other element names.

We compiled from NCBI databases in total 1974 strip of *A.ferrooxidans* protein sequence information, including iron, sulfur, arsenic, copper, cadmium, mercury and other 20 kinds of elements, each element separately related protein sequence information were set aside in the

Excel table. We extract the protein sequence information including registration numbers of proteins serial number, species name, the number of amino acids, and the amino acid sequence of the protein structure, and so on. NCBI database data in a high practicability, but also on the depth and breadth is not able to meet established *A.ferrooxidans* the demand for professional database of all elements related proteins. Therefore, in order to improve the quality of the database, we have access to a large number of documents, in addition to the data in the database references cited, we also expanded the scope of the inspection in accordance with relevant documents, so as to ensure the data elements related proteins A.ferrooxidans Background Information reliability and practicality. According to these documents, we added the 164 protein sequences.

3.2 Database analyses

This home page or search page of the database,this paper summarized the situation of DMRP database, and provides several other important database website links,such as NCBI,DDBJ,GenBank,etc.In addition,*A.ferrooxidans* metal related proteins and the importance of metabolic mechanism research,we are still in the home page lists 9 vice *A.ferrooxidans* related metabolic pathways of graphic, by clicking on the different images,can be more deeply into the specific literature.Home page is the database retrieval page at the same time, comprehensive query service for the data.Over the page, we offer three fields of keyword queries,including protein sequence number number(GI),GenBank(code), the element.We have designed three kinds of logical relationship, for users to choose,including "and,or,not".After the user submits the search request,will get the search result list.Results in the list include protein serial number,registration number,number of amino acids,sources of primary structure,species and the information such as protein sequences.

3.3 Website Structure

3.3.1 Home page

This database of home page that is retrieved, briefed DMRP database, and provides links to several other sites important databases, such as NCBI, DDBJ, GenBank and the like. In addition, the importance of A.ferrooxidans metal-related protein metabolism and research, we are still home page lists the 9 picture of *A.ferrooxidans* related metabolic path, by clicking on the different images to enter specific literature in a more in-depth understanding.At the same time ,the Home page also is the database retrieval , comprehensive inquiry service data. In the top of the page, we offer three keyword search fields, including protein sequence number (GI

number), GenBank (code) elements. After the user submits a search request, you get a list of search results. Results list includes protein sequence number, accession number, the number of amino acids, a structure, species and other information sources and protein sequences.

3.3.2 Browse Page

Browse page provides data on the database by browsing element type classification, behind each element type with the number indicates the number of data entries in this element. It comprises a total of 20 elements and one kind of structure related proteins 2128 cluster data.

3.3.3 Data display page

Detailed data display page lists the details of each data, including basic information about proteins, Such as the registration number, filing date, origin of species, the number of amino acid and protein sequences, etc., we follow Fasta format shows the amino acid sequence. Currently, DMRP site data system has been released, the URL is: http: //www.eamsyslzb.com/Default.aspx.

3.4Analytical procedures

With the rapid development of experimental technologies and proteomics,more and more structures of metal-related proteins will be available(Kucera et al., 2013). DMRP will be updated once every three months. The new protein seq-uence information will be added in time and new analyses will be carried out. It can be expected that our databases will facilitate the research on metal-related proteins.

3.5*Acidithiobacillus ferrooxidans* arsenic response protein evolution analysis

3.5.1 Analysis of basic properties of arsenic - responsive proteins

The physico-chemical properties of 63 *A.ferrooxidans* arsenic-responsive protein sequences in the DMRP database were predicted and analyzed. The sequence of each protein was input into the software ProtParam to obtain a series of predicted physico-chemical properties of the protein, including the number of amino acids, which contained the total number of positive and negative charge residues, Grand average of hydropathicity (GRAVY), and the theoretical pI of the protein.

The proteins are made up 20 amino acids, with different side chains, as a whole or different regions show different hydrophobic characteristics, suggesting that the corresponding structure and function of proteins, commonly used software ProtScale. ExPASy's ProtScale program can be used to calculate the hydrophobicity of proteins. The input data can be the

sequence number of the protein sequence or SWISS-PROT database. In this paper, 63 acidithiobacillus ferrooxidans arsenic-responsive protein sequences in DMRP database were input into ProtScale, and then the results were analyzed to determine the hydrophobicity of the whole protein. The presence of a transmembrane domain suggests that the protein may act as a membrane receptor, or it may be an anchored protein or ion channel protein located in the membrane. On-line analysis of the arsenic-responsive protein transmembrane region was performed using TMHMM Server v. 2.0.

3.5.2 Sequence Analysis of Arsenic - responsive Proteins

We analyzed the sequence information of 63 arsenic-responsive proteins of Acidithiobacillus ferrooxidans by software TMHMM2.0, Protscale and Protgaram, including the total number of positive and negative charge residues, theoretical isoelectric point, average hydrophilicity, and transmembrane Area. Some of these proteins showed identical physical and chemical properties, indicating that these proteins are identical in different subspecies of Acidithiobacillus ferrooxidans and may perform the same function. The theoretical isoelectric point of these 63 arsenic-responsive proteins was 10.63 and the lowest was 5.45. The size of the PI protein is specific, and the structure of the protein. We can isolate and purify these proteins according to the isoelectric point of the protein.

For the analysis of the transmembrane segments, only 4 of the 63 arsenic-responsive proteins showed transmembrane regions, and the 4 proteins contained 8 transmembrane segments, and the positions of the transmembrane segments were all between 25-50, 50-80, 100-130, 147-175, 178-205, 220-250, 256-280, 350-420. Of the remaining 59 proteins, 31 were located in the extracellular domain and 28 in the intracellular domain. For these four proteins with transmembrane domains, further analysis was needed to determine whether they were cell membrane-anchored proteins, carrier proteins, or ion channel transmembrane proteins.

All of the genes of *A.ferrooxidan* ATCC 23270 have been sequenced, but only a small part of the gene function has been confirmed, but a lot of work is needed. With the deepening of research on protein results and biological mechanism, the prediction of protein function becomes more and more important. We analyzed the basic properties of 63 *A.ferrooxidan* arsenic-responsive proteins in the DMRP database, including the analysis of hydrophobic and transmembrane segments, and constructed phylogenetic trees for the proteins involved in arsenic metabolic pathways. And other things in the middle of the evolutionary relationship, in order to better understand the function of these proteins, the need for further protein

structure analysis.

3.5.6Construction of arsenic-associated protein evolutionary tree

Phylogenetic tree is a tree-like branch of the graph, used to describe the relationship between the species. The phylogenetic tree constructed by comparing the values of biomolecule sequence differences is a molecular phylogenetic tree. We selected the arsenate membrane protein and the arsenate resistance protein to construct the respective protein phylogenetic tree. First, the two proteins were first BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) sequence similarity comparison, pick a few similarity of the higher protein, and then from the NCBI to download these protein FASTA format sequence files. Then the molecular phylogenetic tree was constructed by MEGA5.0, and the results were analyzed.

DMRP database of 63 Acidithiobacillus ferrooxidans arsenic response prote-in sequence analysis to predict the physical and chemical properties. The sequ-ence of each protein ProtParam input to the software, it will give a series of proteins that predict the results of physical and chemical properties, including the number of amino acids (Number of amino acids), the total number of posit-ive and negative charge of the protein contained in the residue with the avera-ge hydrophilic (Grand average of hydropathicity, GRAVY), and the theoretical -isoelectric point of the protein (theoretical pI) and the like.

By BLAST search software arsenite resistance protein sequence similarity, I passed analyzed with a collection of 17 sequence phylogenetic tree was const-ructed, these high similarity of isolates from acidophilic *Thiobacillus ferrooxid-ans, Leptospira, pottery Ecuador Escherichia, Burkholderia pseudomallei, fake*

production base aeruginosa, Acinetobacter baumannii, Pseudomonas putida,

Loffi Acinetobacter, Burkholderia more bite, denitrifying Pseudomonas, Ralstoni-a sp.A12*, new onion Burkholderia, Pseudomonas stutzeri, Acidithiobacillus ferri-durans*（Figure 1）.Wherein close affinity with *Acidithiobacillus ferrooxidans*

several acidophilus is by the same branch evolved, high sequence similarity.

And based on phylogenetic tree can be seen arsenate resistance protein and

reducing dependence flavin coenzyme Ⅱ ribonucleotide reductase high sequencesimilarity, these two proteins have a high similarity in amino acid compositionmay their function is also performed by the same, which requires further verifi-cation.

By BLAST search software arsenate membrane pump protein sequence similarity, I passed analyzed with a collection of 13 sequence phylogenetic tree was constructed, these high similarity of isolates from *Acidithiobacillus thiooxidans, Acidithiobacillus ferrivorans, Acidithiobacillus caldus,Thiobacillus prosperus, Thiomonas arsenitoxydans, Acidisphaera rubrifaciens* HS-AP3, *Acetobacter aceti, Acetobacter tropicalis, Gluconobacter oxydans, Xanthomonas oryzae, Aurantimonas coralicida, Gammaproteobacteria bacterium* MFB021, *Psychrobacter, Pseudomonas sp.* 12M76 air, *Halomonas zhanjiangensis, Martelella sp.* AD-3 （Figure 2）. These proteins at the N-terminal and C-terminal high similarity between sequences differ in some large sites, which may differ in their functional areas.

Discussion

In recent years, the bioactivity and related reaction mechanism of bioleaching microbes is one of the most important issues in bio-metallurgy research, which is of great value to understanding the metabolic mechanism of A. ferrooxidans and improving bioleaching efficiency. The current study of *A.ferrooxidans* metabolic mechanism is mainly focused on the extensive and evolutionary relationship of related proteins in the biological world, and the study of the interaction of these proteins. However, a lot of data are scattered in different databases and good literature, which bring further research Many difficulties(Hu et al., 2012). Therefore, the urgent need for the collection and management of these data. In this context, this paper established the first Aferrooxidans metal-related protein database DMRP, and through the integration of network resources and literature mining, increased data depth and breadth, improve data quality and practicality. Users can search and browse the data through the website http://www.eamsyslzb.com/Default.aspx, so that they can provide more support for the metabolic mechanism of bioleaching.

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Table1　63 arsenic-responsive protein sequences of *Acidithiobacillus ferrooxidans*

Protein name	**Asp + Glu**	**Arg + Lys**	**Theoretical pI**	**Grand average of hydropathicity**	**transmembrane region**
ArsH, partial	25	23	6.13	-0.296	outside
30S ribosomal protein S16	8	16	10.47	-0.244	outside
membrane protein in asrC 5'region	32	50	10.00	-0.327	outside
ADP-ribosyl-(dinitrogen reductase) hydrolase	55	51	6.37	-0.130	outside
ADP-ribosyl-[dinitrogen reductase] hydrolase	51	44	5.92	-0.102	outside
unknown, partial	32	50	10.00	-0.327	outside
unknown, partial	7	9	9.67	-0.005	inside
16S ribosomal RNA processing protein	24	12	4.26	-0.006	outside
30S ribosomal protein S16-like protein	8	16	10.47	-0.244	outside
signal recognition particle protein-like protein	57	65	9.38	-0.212	outside
ArsR-like protein	13	11	6.18	-0.280	inside
ArsH-like protein	30	27	5.87	-0.322	outside
arsenical membrane pump	16	24	9.87	1.064	8
arsenical resistance protein ArsH	30	27	5.87	-0.371	outside
NADPH-dependent FMN reductase ArsH	30	27	5.87	-0.371	outside
transcriptional regulator, ArsR	13	11	6.18	-0.280	inside
ArsR family transcriptional regulator	21	26	9.36	-0.114	outside
ArsR family transcriptional regulator	11	14	8.69	-0.027	outside
ArsR family transcriptional regulator	13	11	6.18	-0.280	inside
ArsR family transcriptional regulator	12	14	8.34	-0.017	outside
ArsR family transcriptional regulator	13	15	8.71	-0.238	inside
ArsR family transcriptional regulator	6	14	10.63	-0.026	inside

Protein name	Asp + Glu	Arg + Lys	Theoretical pI	Grand average of hydropathicity	transmembrane region
ArsR family transcriptional regulator	20	20	6.83	-0.200	outside
ArsR family transcriptional regulator	14	11	5.54	0.072	outside
ArsR family transcriptional regulator	15	22	9.44	-0.048	outside
transcriptional regulator, ArsR family	15	22	9.44	-0.048	outside
transcriptional regulator, ArsR family	13	11	6.18	-0.280	inside
putative transcriptional regulator, ArsR family	21	26	9.36	-0.114	outside
transcriptional regulator, ArsR family	12	14	8.34	-0.017	outside
transcriptional regulator, ArsR family	13	15	8.71	-0.238	outside
transcriptional regulator, ArsR family	6	14	10.63	-0.026	inside
transcriptional regulator, ArsR family	20	20	6.83	-0.200	outside
transcriptional regulator, ArsR family	11	14	8.69	-0.027	outside
transcriptional regulator, ArsR family	13	15	8.71	-0.238	inside
transcriptional regulator, ArsR family	6	14	10.63	-0.026	inside
transcriptional regulator, ArsR family	21	26	9.36	-0.114	outside
arylsulfatase	16	24	9.87	1.064	8
arsenical pump membrane protein	16	24	9.87	1.064	8
arylsulfatase	16	24	9.87	1.064	8
arsenate reductase	22	15	5.45	-0.125	outside
arsenate reductase	22	15	5.45	-0.125	outside
ADP-ribosyl-(dinitrogen reductase) hydrolase	51	44	5.92	-0.102	outside

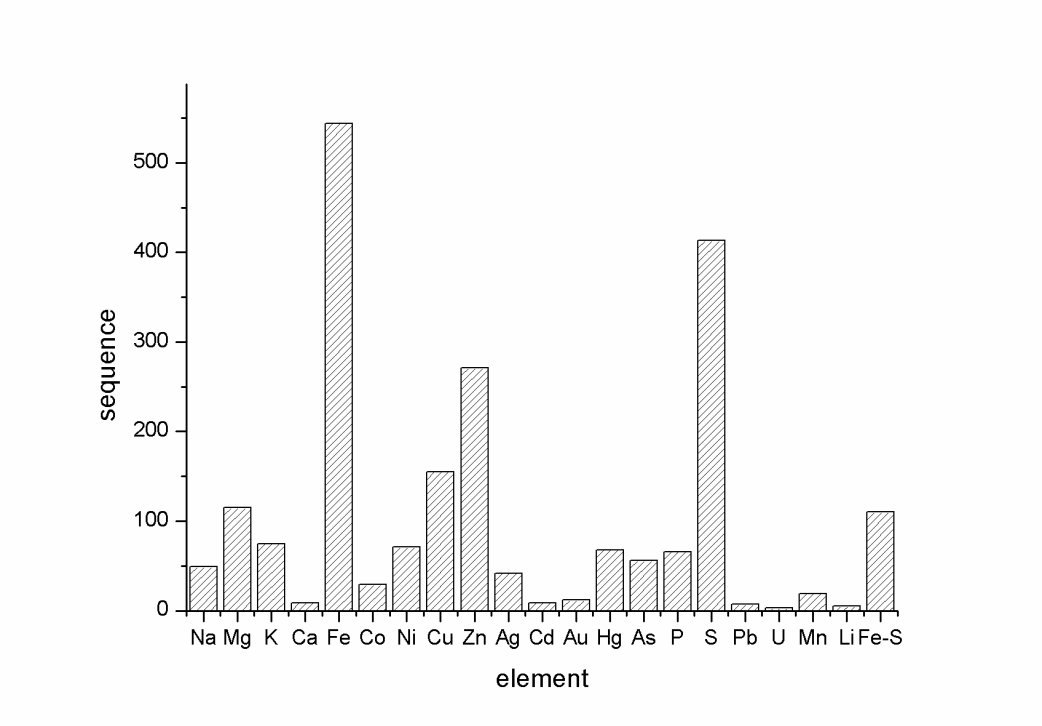

Figure1. The number of DMRP database contains each element in the proteins

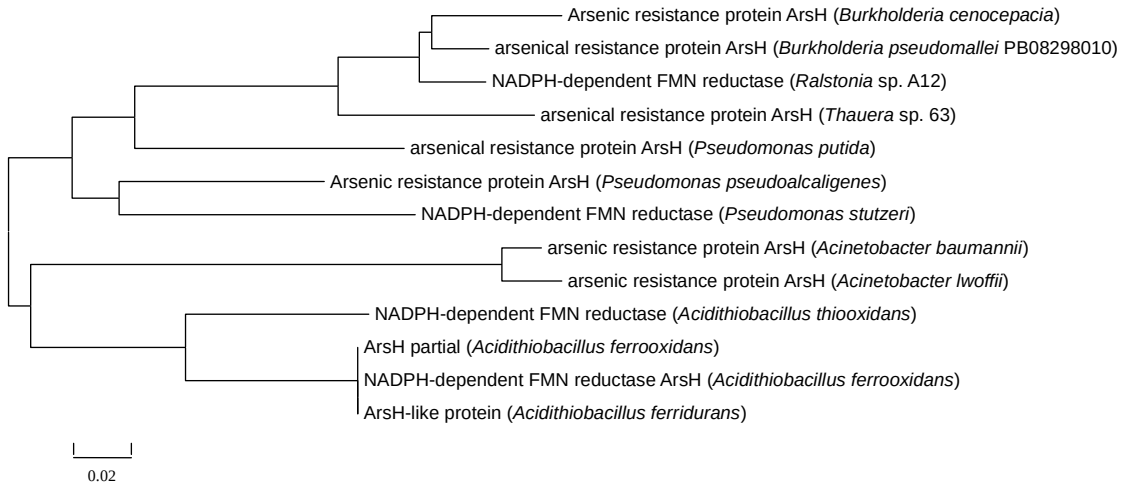

Figure 2. Arsenate resistance protein evolutionary tree

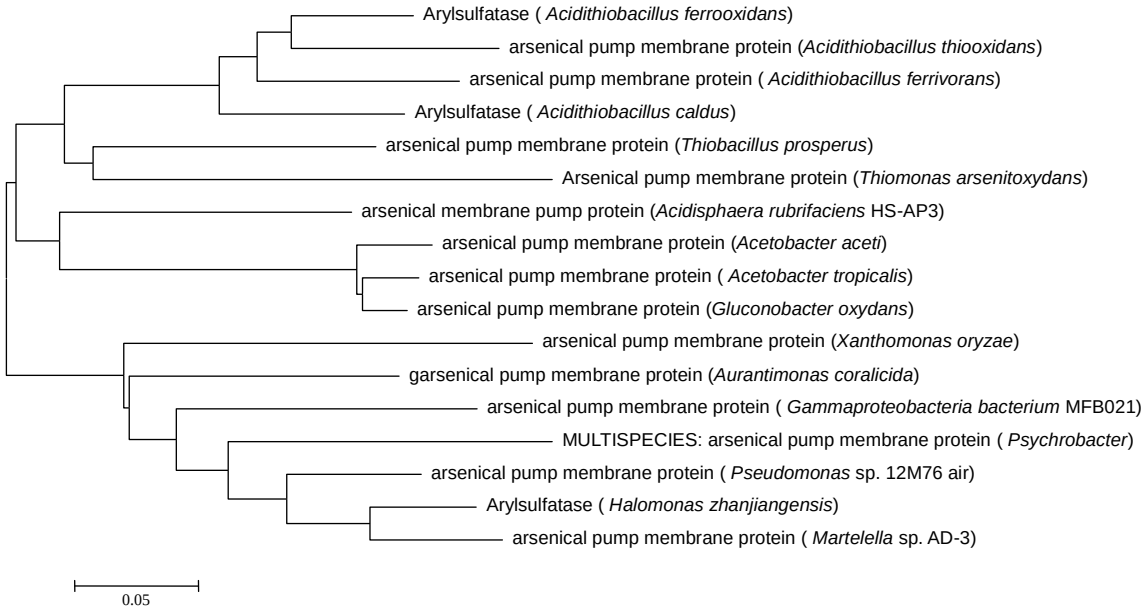

Figure 3. Arsenate pump protein evolutionary tree

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